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Chemical Antibiosis to Nematodes in Rice Fields.

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CHEMICAL ANTIBIOSIS TO NEMATODES IN RICE FIELDS

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ABSTRACT

Nine rice field sites were studied during 1963 and four during the 1964 growing season to test the hypothesis that metabolic fatty acids produced by Clostridium sp. were antibiotic to nematodes under field conditions. Variables measured in soil samples included organic carbon total nitrogen, fatty acids, hydrogen sulfide, and populations of nematodes and anaerobic spore forming bacteria. The results showed that the key factor in the reduction of nematode populations, postulated for mixtures of fatty acids, is hydrogen sulfide.

Fatty acid analyses of soil samples removed from rice fields during the 1963 growing season indicated the presence of acetic, propionic and butyric acids. Peak concentrations of the acids occurred immediately after flooding and again later in the season; in both cases these values declined. Acetic acid was the most abundant acid in the samples followed in order by propionic and butyric acids. Flood waters contained the three acids at volume concentrations approximating 1/15 of those in the soil. Butyric and propionic acids disappeared from rice field soils when added to them; acetic acid concentrations also decreased but to a lesser extent. This indicates a possible transformation of the heavier acids into acetic acid through their utilization by soil microorganisms. Formic acid was absent in all rice field samples.

Additions of corn meal to rice soils in greenhouse studies produced increased amounts of butyric and propionic acids; their combined values

increased exponentially throughout the test and this increase was related inversely to the total number of nematodes at each sampling.

Nematode populations in field plots decreased progressively after flooding. The percentage decrease of plant parasitic nematodes was significantly greater than that of all nematodes combined. Laboratory and field data demonstrated that the decrease in nematode numbers was not related to the acid concentrations in the 1963 field plots.

Hydrogen sulfide proved to be toxic to nematodes in the laboratory at concentrations equivalent to those in rice fields. A marked inverse relationship was obtained in the 1964 season between nematode numbers and the continuous increase of hydrogen sulfide concentrations in rice fields as the season progressed.

Laboratory studies of oxygen concentrations in the rice root zone demonstrated that oxygen is present in the soil at all root-inhabiting depths through the season. Combined evidence indicates that oxygen makes it possible for nematodes and other aerobic organisms to inhabit the rice root zone in soils submerged under water and that this gas exerts a depressive effect against soil anaerobes, other than Desulfovibrio sp. in rice fields.

INTRODUCTION

The soil is a complex matrix in which a variety of processes are continuously taking place in such a manner that a certain degree of equilibrium is maintained. Consequently, a change, however slight, in the soil system results in a reorganization of its component parts to establish a new equilibrium. The variation will in most cases be proportional to the magnitude of the change.

When an aerated soil is flooded, as in a rice field, there occurs a readjustment of component processes caused by the replacement of air by water. It is evident that the formation of new equilibria will be based, at least to some extent, on new processes, typical of more reduced environments resulting from a shortage of oxygen.

The formation of factors antibiotic to nematodes in rice fields (19, 26) is part of the overall reshaping of the soil after flooding. Only those organisms capable of developing under submerged conditions remain in the soil.

The stylet nematode (Tylenchorhynchus martini Fielding 1956) was shown by Johnston (28, 29) to be inactivated by fatty acids produced by a species of Clostridium under laboratory conditions. A number of anaerobic bacteria are known to produce fatty acids as metabolic end products (7). The question investigated in this work was to ascertain their importance in the rice field complex, in relation to nematodes, i.e., were fatty acids produced by bacteria in rice fields in sufficient concentrations to kill nematodes? and if so, for how

long were the fatty acids present in rice fields? These questions were answered and as a result a new component of the submerged soil equilibrium, hydrogen sulfide, was connected with the decline of nematodes (51, 52).

The oxygen content of the rice root zone (50, 76) was investigated in an attempt to answer questions related to a possible effect of lack of oxygen around rice roots on survival of nematodes in rice fields (52).

The discovery that hydrogen sulfide is a nematicidal agent in rice fields opens a new area of research, dealing with the role played by sulfate and iron reducing bacteria, not only in relation to nematodes, but also in connection with physiological diseases of rice and the improvement of cultural practices in the broadest sense.

HISTORICAL REVIEW

I. Reduction of Nematode Populations in Submerged Soils

Reduction of nematode populations in water-saturated soils is an old phenomenon but an understanding of the mechanisms involved has been lacking. In 1911, Bessey (6) observed in the Southeastern U.S.A. that flooding reduced the amount of damage due to nematodes and recommended that land be flooded for at least twenty-five days. Frandsen (13) concluded in 1916 that at least three months submergence would be needed to make the method effective. Watson (79) in 1921 was optimistic about flooding as a means of controlling root know (Meloidogyne sp.) in Florida. Perhaps the first observations on the decline in nematode populations in rice fields were those of Imamura (24), who reported a reduction in the number of nematodes with flooding in the paddy fields of Japan. This worker made studies on the nematode species of rice fields and noticed that some of them were more drastically affected than others by flooding. The reaction of nematode species to fluctuations in soil water has been studied in some cases quite extensively. Wallace (78) noted that most species of nematodes were adversely affected by high soil moisture but that some, like Radopholus oryzae (v. Breda de Haan, 1902), Filipjev, 1936, inhabit paddy fields where they were parasitic on rice.

Hollis and Fielding (20) reported a correlation between fluctuations in populations of T. martini and soil moisture changes produced by intermittent

flooding. High numbers occurred in Crowley silt loam rice plots that had not been flooded with water and low numbers occurred in plots that had been flooded.

The drop in nematode populations consequent with flooding had been observed repeatedly and it was commonly assumed that anaerobic conditions in the soil (13), and exhaustion of the nematodes due to increased activity in water (8) or drowning (24) accounted for such reductions. In the light of these theories the possibility of nematode death through a lack of oxygen in the submerged environment would be of primary importance.

In 1954, Feldmesser (9) studied the effect of altered oxygen tensions on certain plant-parasitic and soil inhabiting nematodes. He reported that species of Aphelenchoides, Heterodera, Meloidogyne, and Rhabditis, while exhibiting a progressive loss of motility upon removal of oxygen, recovered invariably minutes after they were returned to normal conditions of aeration. More recently, Van Gundy, Stolzy and coworkers (58, 74, 75) conducted laboratory tests to determine the effect of low oxygen tensions in soil on root-knot nematodes and other species. These workers were able to show a direct relationship between decreasing oxygen concentrations in soil and survival of the nematode species tested. These tests, however, were performed in soil which could introduce other variables; at low oxygen tensions populations of anaerobic microorganisms quickly develop in wet soil and their metabolic products could be toxic to nematodes (75, 81).

The problem of fluctuation in T. martini populations (20) was taken to the laboratory by Hollis and Johnston (19). They observed that results similar to

those observed in field samples could be obtained under laboratory conditions by submerging Crowley soil. T. martini were incubated in sterilized moist soil, nonsterilized moist soil, sterilized water saturated soil and nonsterilized water saturated soil. Consistently lower survival occurred in the nonsterilized water saturated soil. Microbial action against nematodes was demonstrated.

Johnston (27) found the optimum levels of moisture in soil for the survival of T. martini lay between 40 and 60 per cent of field capacity and the survival was lowest at 11 per cent of field capacity and at saturation. Since lowest survival consistently occurred at submerged levels, the possibility of the action of an anaerobic organism or organisms was emphasized. In another paper, Johnston (26) was able to show that reduction of nematodes was greater in non-sterilized soil without oxygen than in sterilized soil without oxygen. He concluded that whatever the mechanism controlling nematode reduction, it was favored by anaerobic conditions and that the lack of oxygen was not the cause of such population reductions. This resulted in the isolation of a species of Clostridium which produced a substance toxic to T. martini when incubated in artificial culture fluids. The species of Clostridium was identified as Cl. butyricum, Prazmowski, 1880. The effect of this bacterium on populations of T. martini was studied by Johnston (28), who found that when the nematode was introduced into sterilized soil enriched with corn meal in the presence of Cl. butyricum its population was significantly decreased after 3 days incubation and was reported to be nil after 6 days incubation. Analysis of culture filtrates revealed the presence of formic, acetic, propionic, and butyric acids, all of

which were found to be toxic to T. martini. Inactivation of the nematodes by a mixture of the 4 acids at concentrations found in the culture filtrates occurred 12 hours after initial exposure but the individual acids required a longer period of time to inactivate the nematodes. A study on the effectiveness of mixtures of the 4 acids on the nematodes at the concentrations found in culture filtrates showed that any combination containing butyric acid was effective (29). The action of some fatty acids against nematodes had been previously studied by Tarjan (70, 71), who found that butyric acid was active against nematodes but that not all nematodes responded alike when exposed to water solutions of this acid.

Johnston's studies indicated a possible explanation for the nematode decline observed in rice fields; however, it was necessary to corroborate his results under actual field conditions. To confirm his findings it was necessary to determine the kinds and amounts of these acids normally found in rice fields, the amount of time that these acids remained in the field as the season advanced, and to determine the nematocidal effect of the acids at field concentrations. This information could then be checked against nematode counts in the field through the season.

II. Fatty Acids in Soils: Their Kinds and Amounts

The lower fatty acids are characteristic metabolic end-products of the anaerobic decomposition of organic matter in waterlogged soils (2, 77); their presence in poorly drained soils and soils under submerged conditions has

stimulated a number of studies of flooded soils under laboratory conditions to which some form of organic matter is added (2, 47, 48, 81). Subrahmanyan (61, 62) in studying the decomposition of different carbohydrates in flooded soils under laboratory conditions noted that lactic acid first appears after soil submergence but that it decomposes readily to form acetic and butyric acids; he also reported that the decomposition of lactic acid was favored by the addition of calcium carbonate to the soil. Acharya (1) studied the decomposition of rice straw with soil under flooded conditions. He observed that the first phase of the decomposition was the rapid formation of organic acids. A second stage involved the decomposition of the organic acids with the formation of methane and was more sensitive to acidity than the first phase. The products of the decomposition of rice straw were acetic acid, butyric acid, carbon dioxide, methane, and negligible amounts of hydrogen.

The lower fatty acids have been repeatedly demonstrated in both aerated and waterlogged soils by different workers (17, 39, 40, 54, 61, 62, 67); Schwartz, Varner, and Martin (54) analyzed Ohio soils with varying organic matter contents and obtained acid concentrations ranging from 0.02 m.e. to 1.08 m.e. per 100 grams of soil. The concentration of the acids seemed to be directly related not only to the total carbon in the soil but to the total nitrogen as well. The main acids in these well aerated soils were acetic and formic acids, with 2 groups of unidentified acids. The authors also found an initial rise of acetic acid with a concomitant lowering of formic acid concentrations, however no explanation of this phenomenon was advanced. Takai (66, 67) in a

study with different Japanese rice soils found concentrations of lower fatty acids varying from 0.03 to 2.36 m.e. per 100 grams of soil. These studies, while indicating concentrations of lower fatty acids to be expected in rice fields are of little value otherwise, since sampling was performed only a few times after flooding and was not continued throughout the entire rice growing season.

III. The Occurrence of Sulfides in Submerged Rice Soils

Laboratory tests performed during the course of this research revealed hydrogen sulfide as a nematicidal agent (52); the significance of this discovery resulted in the determination of sulfides during the 1964 rice season in an attempt to evaluate the antibiotic action of these compounds against nematodes under Louisiana rice field conditions.

The anaerobic conditions resulting from flooding of soils enhance the formation and accumulation of sulfides in soils, often in excess of 150 ppm (2). A distinct black zone containing abundant ferrous sulfide is oftentimes deposited in the soil profile (47). The mineralization of organic sulfur in soils under submerged conditions affords another source of sulfides (47).

The formation of sulfides in waterlogged soils is mainly due to the activity of Desulfovibrio desulfuricans (Beijerinck, 1895) Kluyver and van Niel, 1936. Morphologically the cells are curved rods rendered motile by means of a single polar flagellum (7). These bacteria while being strict anaerobes can tolerate oxygen in their medium due to the high concentrations of sulfides which quickly form around the black colonies (47, 57).

The presence of sulfides in rice fields has been repeatedly demonstrated in the past (17, 35, 39, 40, 42, 43, 44, 60, 63, 64, 65, 68, 69). Takai (64, 65) described a period of active sulfide formation in some Japanese soils after considerable time of incubation; this was preceded in the earlier stages of flooding by a rapid decrease in oxidation-reduction potentials of the soil and by the disappearance of nitrate nitrogen with a concomitant liberation of ammonia from the soil. Aerobic bacteria reached their highest numbers soon after flooding, followed by an increase in anaerobic bacteria which were then replaced by the sulfate reducing group. This worker observed a continuous increase in sulfides in the flooded soils from initial values of 1 and 2 ppm to as high as 24 and 62 ppm after 21 days of submergence. Sulfate reducing bacteria increased correspondingly from 0.3 and 0.4 million per gram of soil to 2.3 and 1.7 million per gram of soil in the two soils used by this worker. More recently Mandal (35) in laboratory studies on the transformations of iron and manganese in waterlogged soils of India obtained similar results; the concentration of hydrogen sulfide increased from 0 to 15 ppm through a 78 day period of submergence. Treatments with green manure and straw supplements resulted in higher levels of hydrogen sulfide in the same submergence period.

Aomine (4) in a review of studies on the oxidation-reduction potential of Japanese soils indicated the relationship between low O-R values and the presence of sulfides and reduced iron and manganese, previously suggested by Sturgis (60).

The formation of free hydrogen sulfide has been linked to the Akiochi

disease of rice (37, 42, 43, 44) and the possibility of a connection between the presence of this gas in rice fields and the Mentek disease of rice in Java cannot be ignored (60. 73). Hydrogen sulfide has been suggested recently as a possible factor in the flood fallow control of fusarial wilt of bananas in Honduras (59).

Rodriguez and Jordan (51) proposed a mechanism for the production of molecular hydrogen sulfide in rice fields; the lower fatty acids present in the flooded fields were visualized as acting on the metallic sulfides deposited in these soils with the consequent formation of molecular hydrogen sulfide and soluble metallic salts of the acids. The reaction of weak acids on sulfides had been previously described by Treadwell (72). Conditions promoting these reactions lie within the range of hydrion and fatty acid concentrations found in the soil.

MATERIALS AND METHODS

I. The Choice of Representative Experimental Sites

The Louisiana rice area comprises a total of approximately one million five hundred thousand acres, a third of which is under cultivation every year owing to the three year rotation normally followed by the growers. Rice is grown in this state, principally, as far South as Cameron, Vermillion, St. Mary and Terrebone parishes and North to Vernon Parish. Soils in this area range from silty clay loams to more predominant silt loams. These soils are characteristically low in total nitrogen and organic carbon and generally respond to additions of nitrogen fertilizer and in some cases to additions of phosphorus. The subsoil is impervious and composed of heavy clays.

Due to the diversity of soil types which are grown to rice in Louisiana as well as the varying ecology of different fields, it was thought essential for this work to choose a number of plots in a random fashion throughout the area so as to insure an effective representation. A total of nine sites were set up on this basis for the 1963 rice season; each of these sites was divided into two 20 x 20 feet plots; in each case one of the plots received the equivalent of 8.6 tons of cotton seed hulls per acre (A plot) and the other was left as a check (B plot). The four sites chosen for the 1964 season were composed of individual 10 x 10 plots and received no cotton seed hull treatment. The 1964

plots were essentially in the same location as those corresponding for the previous year. Table 1 shows the cooperator, location and soil types of each rice field site.

These nine sites were sampled intensively through the 1963 season whereas only the Davis, Richard, Caffey and Wild sites were used in the 1964 experiments.

II. Soil Sampling Technique

Soil sampling was conducted in duplicate 10 x 10 feet plots at the selected locations. Soil samples were composited from cylindrical cores of 4.3 inches length and 3 inches diameter, taken at random from the plots and then packed and sealed in quart jars. Single cores were removed to pint jars for pH and Eh determinations.

III. Soil Moisture Determinations

Soil moisture was determined by placing a sizable portion of the wet soil at every sampling in tin cans one and three tenths inches high by four inches in diameter. The cans were then placed at 105°C for twenty-four hours in an electric oven. The percentage moisture was then determined by differential weighing and referring to the oven dry soil.

IV. Determination of the Hydrogen Ion Concentration and of the Oxidation Reduction Potential of Soil Samples

Hydrogen ion concentration was determined by inserting glass electrodes

Table 1. Location and characteristics of rice field experimental sites in 1963.

Cooperator[1]	Soil Type	Location of Site
Richard Brothers	Acadia-Wrightsville Silt Loam	N. E. of Kinder
Floyd Compton	Crowley-Midland Silt Loam	N. E. of Roanoke
C. M. Davis	Crowley-Midland Silt Loam	North of Jennings
E. E. Wild	Midland-Crowley Silt Loam	South of Midland
Lyle Fogelman	Crowley Silt Loam	S. W. of Crowley and North of Bayou Queu de Tortue
E. Stansel	Mucky Clay Fresh Water Marsh	South of Gueydan
M. Byler	Crowley-Midland Silt Loam	West of Thornwell
H. R. Caffey	Midland Silt Loam	L. S. U. Experiment Station at Crowley
A. Petitjean	Jeanerette Silty Clay Loam	N. E. of Rayne

[1]The names of the cooperators are used in this work to refer to the individual sites.

from a properly standardized Beckman Zeromatic pH meter adjusted to a standard temperature of 25°C and maintained in the laboratory.

Eh of the samples was determined with platinum and calomel reference electrodes left in the soil for thirty minutes before a reading was attempted. It was found that if the electrodes were left in the soil for this period the Eh of the sample exhibited very little change after thirty minutes.

The platinum electrode was periodically cleaned in a normal hydrochloric acid solution by connecting it to a radio battery (15-25 volts) and using a carbon rod as an anode. The platinum electrode was then subjected to this treatment for three minutes.

Mixtures of 0.1 M citric acid and 0.2 M disodium phosphate buffer solutions ranging from a pH of 2.8 to 8.0 were used for testing and standardization of the electrodes (9).

V. Bacterial Studies: Their Identification and Counting in Soils

Ten grams of soil were used at each sampling to study the numbers of anaerobic spore forming bacteria, as well as to provide cultures for their identification. The soil was transferred in every case to a sterilized 50 ml porcelain mortar, where it was thoroughly ground and mixed with sterile water to give a final volume of 100 ml. These suspensions were then put into sterile 250 ml Erlenmeyer flasks and subjected to a temperature of 80°C for 10 minutes. The flasks were then allowed to cool until a temperature of 40°C was attained.

Serial dilutions from 10^{-1} to 10^{-11} were prepared from the heated soil suspension in 5 replicate test tubes containing a medium of the following composition in gms per liter: soybean hydrolyzate (NBC) 5, potato slices 100, agar 1, dipotassium phosphate (3 H_2O) 1, magnesium sulfate (7 H_2O) 0.2, sodium chloride 0.01, ferrous sulfate (anhydrous) 0.01, manganous sulfate (H_2O) 0.01, glucose (anhydrous) 20. Reaction of the medium was adjusted to pH 6 since this figure represented the general average soil pH for all samples. The use of potato infusion and a high glucose level in this medium was designed to favor the growth of anaerobic starch fermenters which were directly connected with the production of fatty acids.

The tubes once inoculated were placed in ten liter capacity desiccators with alkaline pyrogallol in the proper proportion and two pads of activated iron wool according to the method described by Parker (45). The purpose of these pads was to provide an effective oxygen scavenger in case a leak occurred in the seal of the containers. Owing to a temporary shortage of pyrogallol in the 1963 season, germinating oats were used as substitute. In all cases however, the methylene blue indicator (56) was carefully examined for the decoloration which it characteristically presents under anaerobic conditions. A saturated solution of sodium bicarbonate was also included in all chambers to restore any of the carbon dioxide that might have been absorbed by the alkaline pyrogallol and the activated iron wool.

A ten day incubation time was observed for all determinations after which the anaerobic chambers were opened and the tubes examined for growth. The

numbers of bacteria were determined in accordance with the methods outlined by Halvorson and Ziegler (15, 16).

Five-tenths ml of inoculum was pipetted out of each tube into a fresh tube containing the same medium, in what was considered an enrichment step. These new tubes were then placed under similar anaerobic conditions and the ten days incubation period was again observed. Streak plating was then attempted on a solid form of the medium in Petri plates using inoculum from the enrichment tubes. These plates were placed in chambers for 15 days, after which they were opened and individual colonies were transferred to tubes filled with a modification of the isolation medium containing 10 grams of calcium carbonate (31, 49). These tube cultures were then subjected to anaerobic conditions for 10 days to provide for proper development of the bacteria after which microscopic examination of the individual cultures was made. Where doubts arose as to the purity of the isolate, the procedures described were repeated until purity was attained. These isolates provided the stock cultures on which microscopic observations and physiological tests were carried out for identification, in accordance with the taxonomic keys presented in the seventh edition of Bergey's Manual of Determinative Bacteriology (7).

The isolation of Desulfovibrio sp. from field samples was made in the 1964 rice season according to the procedures of Allen (3) on both liquid and solid Van Delden's media.

VI. Extraction of Nematodes from Soils

Nematodes were extracted from 200 ml samples of soil using procedures recommended by Seinhorst (55). The nematode suspension was collected on a triple 300-mesh screen, then washed into a Petri dish fitted with a plastic cylinder containing a screen bottom covered with "ederol" filter paper. After 24 hours incubation, the sample was transferred from the Petri dish to a Syracuse watch glass ruled to permit counts of 1/7 sample aliquots (20).

VII. The Analysis of Organic Carbon and Total Nitrogen in Soil Samples

The organic matter of the soil samples was determined as elemental carbon following Walkley and Black's rapid titration method described by Piper (46). In all cases at least three replicate titrations were made of each sample and the final result expressed as the average. Total nitrogen determinations were made in duplicate from every sample following a modified Kjeldahl method described by Jackson (25).

VIII. The Extraction of Fatty Acids from the Soils

The lower moncarboxylic fatty acids were extracted from the soil by taking 30 grams of water-saturated soil or 20 grams of air dry soil in a modification of the method developed by Takai and coworkers (66, 67). The soil was divided into two portions in two 50 ml "lucerite" tubes, to each of which 30-40 ml of distilled water was added and mixed thoroughly with the soil. The

tubes were then centrifuged at 3000 RPM for 30 minutes, after which the supernatant was collected. The centrifugation was repeated twice with an additional 30-40 mls of distilled water, so that three supernatants were obtained from each soil. The total supernatant fluid was then passed through glass columns 60 cm long x 9 mm internal diameter, containing 10 ml of Amberlite (IR-120) H type ion exchange resin, to adsorb basic substances and inorganic cations. The eluent was collected and neutralized with N NaOH to a phenol red end point in 600 ml Pyrex glass beakers. The solutions were then placed in an electric oven at 60-80°C and evaporated to dryness. The salts obtained in this manner were then dissolved in small amounts of distilled water and successively transferred and dried in smaller containers until all of them were finally collected and left in the dry salt form in 2 ml glass vials. The acids were kept as the dry sodium salts until gas chromatographic analyses were carried out to resolve the mixtures of acids. At this time 1 ml of N HCl was added to the vials to bring the free organic acids into solution.

IX. Analysis of Fatty Acids Extracted from Soil Samples

A MicroTek 2500R unit for gas-liquid chromatography employing flame-ionization detection was used for the quantitative determination of fatty acids in the C1-C5 range. Columns were packed with 20 per cent tween 80, 2 per cent phosphoric acid (W/w) on 30-60 mesh acid-washed chromasorb w, according to the procedure of Gehrke and Thornton (14). The column temperature was maintained in all cases below 130°C. Helium was used as the carrier

gas with the flow controlled at 75 ml/minute. The inlet block temperature was kept at 180°C, the outlet block temperature and the flame detector block at 190°C, air flow was kept at 300 ml per minute and the hydrogen flow at 40 ml per minute. Aliquots of the samples in 2 microliter quantities were injected into the apparatus for resolution. Each analyses took approximately 20 minutes for the complete resolution of the mixture.

A series of standards were prepared of mixtures of formic, acetic, propionic, and butyric acids in distilled water. These standard solutions were prepared by weighing the pure acids and verified by their titration with NaOH. The standards varied from 0.04 m.e. to 1.0 m.e. per ml for total acids. The total number of milliequivalents in each standard were equally distributed among the four acids.

Results of the analyses were graphed on a Texas Instruments WD recorder. The area of each curve representing an acid was measured with a planimeter and the amount of each of the acids calculated by comparison with the areas obtained for the corresponding acids in the standard solutions.

X. Determination of the Hydrogen Sulfide Content of Soils

Determinations of H_2S in the range of 0.1-40 ppm were made with a polarograph constructed for the purpose. Theoretical details and practical limitations were in agreement with a technique outlined for measurement of hydrogen sulfide in water associated with petroleum deposits (18). A series of standard H_2S concentrations established by iodimetric titrations (Fig. 11)

show the limits of sensitivity of the method, which by extrapolation, can be lowered to 0.01 ppm H_2S. Duplicate 500 ml quantities of water-saturated soil from each sampling were diluted with distilled water, connected to an alkali trap and heated to boiling for 15 minutes. The HCl was trapped in 100 ml of NaOH in flasks, which were then stoppered and held for hydrogen sulfide determinations. Certain alterations of the usual polarographic techniques had to be made in order to detect small concentrations of H_2S. The streaming mercury electrode was employed in order to eliminate the periodic fluctuations in current characteristic of the dropping mercury electrode. Voltage increase was applied at an exponential rate in order to expand the sulfide wave so that it could be more easily interpreted. No maximum suppressor such as gelatin was necessary at low concentrations, but the method proved to be inadequate above 35-40 ppm H_2S, due to abnormal increase in current and the precipitation of some mercury and HgS before the oxidation potential of $S^{=}$ was reached.

All samples were swept vigorously with nitrogen and then kept under an inert atmosphere during analysis in order to eliminate interference caused by oxygen. A relatively high concentration of NaOH (normal) was necessary to provide conditions favoring the formation of $S^{=}$ in the equilibrium between HS^{-} and $S^{=}$. Analysis of H_2S concentrations above 35-40 ppm was attempted after suitable dilution with NaOH, of the same normality as that of the original trap solution, but the presence of interfering substances, believed to consist of humic acid and its fractions, produced erratic results in many of these samples.

XI. Nematodes Used in Laboratory Experiments

Swarming populations of T. martini (21, 22) were employed in laboratory assays of the effects of hydrogen sulfide and fatty acids on nematodes. Their rapid movements in swarms rendered them particularly useful for toxicity tests in replicate 5 ml sealed vials, where they were observed directly. Removal to open dishes was also necessary because lethal concentrations of chemicals inhibit movement prior to kill. Swarming populations of T. martini are of uniformly high vigor, as evidenced by their survival and continued movement in check vials of water for intervals up to two months, but numerous tests have indicated they are equivalent to the more common nonswarming field populations of T. martini in resistance to toxicants (23).

EXPERIMENTAL RESULTS

I. Variations in the Amounts of Organic Carbon in Soil Samples from Submerged Fields During the 1963 Rice Season

The amounts of organic carbon present in samples taken from the nine 1963 sites was determined throughout the rice season at every sampling. The purposes of such analysis was to obtain a clearer picture on the nutritional potential of the soils as related to bacteria producing the fatty acids in the flooded soils and to find if there was a correlation between organic carbon values and the decline of nematodes in submerged rice fields.

The method used for the determination of organic carbon (46) measures not only that part of the organic matter in the soil which is directly utilized by the anaerobic bacteria studied in this research but also other forms of organic matter such as cellulose, lignin, humus complexes that are more difficultly utilized by soil microorganisms. Materials commonly found in submerged soils (2) such as cellulose and lignin are utilized very slowly by the soil microflora under waterlogged conditions (2). Hence, an evaluation of the soil organic matter directly available to the anaerobic spore-forming clostridia of the butyric acid group would be clouded by the presence of those other more abundant forms of organic matter, which are hardly if at all available to the soil microflora of a submerged soil (77). Since the amount of nonavailable soil organic matter remains essentially constant through a period of submergence, any fluctuation

detected in the values of organic matter in a submerged rice soil as the season advances could be attributed only to variations in the amounts of readily available organic matter. Consequently, fluctuations in soil organic matter in rice soils presumably can only be detected in those soils in which the available part of their organic matter is relatively abundant prior to flooding. Since total organic carbon was determined small variations in organic carbon values are important and must be examined in relation to other variables.

Organic matter in a submerged rice soil should be expected to decline as the season advances, only reaching a constant level after its nutritional value has been exhausted. However, in some of the soils organic carbon increased as the rice plants developed. This is presumably due to increments of rice roots, dead leaves, and organic debris included in the samples taken. The general picture of organic carbon obtained from samples taken in the nine experimental field sites during the 1963 rice season was one of decline as the time of submergence increased. However, this soil variable more than any of the others studied in this work, necessitates discussion of results obtained in every field site (Table 2).

I. Fogelman. The percentage of organic carbon in both of these plots decreased from the first sampling 2 days after flooding up to the last sampling 16 days after flooding. This coincided with an increase in the total amount of fatty acids and a decrease in the number of anaerobic spore-forming bacteria. The decrease in organic carbon is related to utilization by the bacteria of

available forms of soil organic matter and supports the above theory about variations in organic carbon values.

2. Davis. The organic carbon values obtained from plots in this soil were rather constant throughout the season. The first sampling, 10 days after flooding may have been too late to detect the utilizable organic matter. This view is supported by the comparatively constant values obtained for both fatty acids and bacterial numbers during the season.

3. Caffey. This site had been under continuous rice culture for three previous years and did not receive organic matter supplements during this time, excepting the A plot which received the cotton seed hulls during the fourth and current year. This supplement, high in cellulose, was applied in the Fall of 1962, and was subjected to aerobic conditions during the winter. Under such conditions cellulose and similar materials decompose faster than in a flooded soil (47). The amounts of organic carbon available to anaerobic bacteria after flooding was thus higher in the A plot than in the B plot, as were the amounts of acids produced. However, as in the Davis plots, most of the available organic matter had already been utilized by the time of the first sampling at 10 days after submergence and values obtained for organic carbon and bacteria in these plots were relatively constant throughout the 82 days of this experiment.

4. Richard. The percentage of organic carbon in the A plot was higher throughout the 1963 season than in the B plot. Organic matter decreased in both plots from the initial sampling up to 89 days after submergence. This

continuous decrease in organic matter throughout the season indicates that a considerable amount of it was in a form available to the anaerobic bacteria. This view is confirmed by the relative stability of the bacterial population in the first 69 days of submergence. The greatest drop in bacterial numbers for the 2 plots occurred at the last sampling and coincided with the greatest decline in organic carbon and the late season increase in organic acids. Organic carbon in this soil followed the general trend of decline observed for soils high in utilizable organic matter. The effect of additions of cotton seed hulls to the A plot in this site was less than in the Caffey plot which had been subjected to continuous rice culture for three previous years. The addition of cotton seed hulls to this depleted soil presumably resulted in a greater, although temporary, increase in production of fatty acids. Soils already high in utilizable organic matter, such as the Richard soil, responded to a lesser extent to supplements of cotton seed hulls.

5. Compton. Organic carbon increased consistently from the first sampling 9 days after submergence and finally decreased to a low value at the last sampling. Available or utilizable organic matter in this soil was probably low at the time of the first sampling. This correlated with the progressive decline in amounts of fatty acids and in the bacterial population.

6. Wild. Organic carbon levels were relatively constant for both A and B plots. Utilizable carbon was low and correlated well with the values for anaerobic bacteria and fatty acids.

7. Petitjean. Organic matter available to the anaerobic spore forming bacteria in this plot was low, in view of the consistent levels of total organic carbon during the 48 day sampling period. This was related to a slow and progressive decline in the population of bacterial anerobes in this soil. Fatty acids increased in amounts throughout the sampling period, but rates of increase were low and probably dependent on the rate at which unavailable organic carbon became available.

8. Stansel. This soil was abnormally high in organic matter, but the constant level of total organic carbon, after the high initial value, 9 days after flooding, indicated low amounts of available organic matter. This view was confirmed by the size of the bacterial population which remained rather constant throughout the season.

II. Total Nitrogen in Flooded Rice Fields During the 1963 Rice Season

Although some of the soil nitrogen is lost from fields under submerged conditions this loss is generally limited to nitrates through their reduction to ammonia, which then escapes from the soil (36, 53). Furthermore, this loss is confined to the first few days after flooding (64, 65); nitrogen losses after such time are beyond the limits of detection by ordinary methods of analysis (2).

The values of nitrogen in per cent obtained from soil samples removed from the nine experimental sites during the 1963 rice season are summarized in Table 2. In general the values were rather constant from all soils throughout

the entire period of submergence although in some soils a slight decrease in nitrogen was detected.

III. Variations in Hydrion Concentration and Oxidation-Reduction Potential of Soil Samples from Submerged Rice Fields During the 1963 Rice Season

The hydrion concentration in soils submerged under water has been repeatedly demonstrated to decrease as the time of submergence increased (48). The elements of this pattern can be related to the growth of rice and to the microbiology of submerged soils (4). It has been reported that an increase in pH is adverse to the presence of organic acids in rice fields (4), however, increases of pH during the 1963 rice season were accompanied by consistently maintained levels of fatty acids in soil samples taken from the nine experimental field sites (Tables 2, 3).

The increase in pH could not be correlated with a decrease in Eh values, as has been reported in other cases (4). Eh values were erratic (Table 2). However, in all cases, reduced conditions (below 200 millivolts) were attained during the first 10 days of submergence and were maintained throughout the entire season. The levels of reduction however, varied in the different soils. Those in which pH values were lowest (Stansel) showed higher Eh values than those in which pH values were higher. Thus, no strong correlation was observed between Eh and pH values but a loose association was detected in individual cases. Correlations were not observed between Eh and pH and other variables measured in the course of this work.

IV. Fluctuation of Fatty Acid Concentrations in Rice Fields

A. Variation of total fatty acids.

The fatty acid data obtained from rice field samples in 1963 suggest a general pattern of behavior for the acids, i.e., an initial rise in total acid concentration followed by a decline and then an increase again late in the season to a high concentration (Fig. 1, 2). The time of occurrence of these high and low values do not always coincide in all of the soil sites but the trend is marked in those fields where a sufficient number of samplings were made. This pattern of behavior becomes clear when results from the individual sites are examined for variation in total acid concentrations (Table 3).

1. Fogelman. Plots were sampled for acids at 3 different times in 1963, beginning 2 days after flooding. There was a negligible amount in both the A and B plots at the initial sampling. However, 7 days later total acids increased tremendously, reaching peak values of .04058 and .04328 m.e. per 30 gms of water saturated soil for the A and B plots, respectively. The third and last sampling at 16 days after submergence showed a significant decline in acid concentration in the two plots. Although no further samples were taken from this site, the initial rise in concentration was observed as well as the subsequent decline in acids.

2. Davis. This site was sampled for acids 4 times during the 1963 season. Ten days was allowed to pass after flooding before samples were gathered from the two plots, consequently the first values were rather high. Concentration increased in the second sampling at 18 days after flooding,

reached a low point 32 days after inundation, and then increased again in both the A and B plots in the final sampling at 52 days after submergence of the soil. Concentration of the acids as a whole showed two maxima, the first at 18 days and the second at 52 days after flooding.

Highest values for initial maxima were .10956 and .06484 m.e. per 30 grams of water saturated soil, for the A and B plots, respectively. Corresponding values for the late season rise in acid concentration were .03156 and .02540 m.e.

3. Caffey. This soil exhibited the same trend in fatty acid concentration as the others. Plots were sampled at 5 different times starting 7 days after flooding of the soil. Total acid concentration in the A plot increased between the first and second sampling and then declined at 32 days and rose again at 62 days after flooding, followed by a final drop 82 days after flooding. The B plot showed the characteristic maxima in acid concentration, but differed from the A plot in the time at which they appeared. The initial maximum concentration was not evident in the B plot until the third sampling and the late season rise in concentration did not occur until the final reading at 82 days.

4. Richard. These plots were sampled 5 times during the 1963 rice season and acid concentrations for the B plot are shown in Figure 1.

5. Compton. This soil was sampled 5 times from 9 to 71 days after flooding in the 1963 rice season. Initial readings 9 days after submergence showed a high concentration of total acids in both the A and B plots. Concentrations in the A plot decreased continuously from an extremely high initial

reading to the final sampling 71 days after flooding. The B plot showed the early rise in acid concentration followed by the usual decline and then increase again, although not to the same level as in the early part of the season. This second maximum was followed by a decline in acid concentration at the final reading 71 days after submergence. Acid values in the A plot declined continuously throughout the season, but they did not reach the low value shown by the B plot at the 30 day sampling, and there was less fluctuation in that acid concentration in the A plot than in the B plot.

Highest total acid concentration in the B plot were .0580 and .04744 m.e. per 30 grams of water saturated soil, corresponding to the early and late season acid peaks. The initial value for the A plot was the highest, .07060 m.e. per 30 grams of water saturated soil.

6. Wild. Plots in this site were sampled 5 times throughout 43 days of submergence. In general, total acids followed the trend shown by the other sites, i.e., of an early and a late season rise. Fluctuations in acid values in the B plot were less pronounced than those in the A plot, particularly in regard to the late season increase. The timing of the increase and fall in concentrations did not coincide at the late season maximum either. However, these plots fell within the general pattern of fluctuations. Highest values for the B plot were .04004 and .04180 m.e. per 30 grams of water saturated soil for the early and late season maxima, respectively. Those of the A plot were .03592 and .01088 m.e. per 30 grams of water saturated soil, respectively for the initial and final rise in total acid concentrations.

7. Byler. This site was sampled at 59 and 72 days after submergence. Acetic acid was present in both of the plots in quantities comparable to those obtained in other soils at similar times after flooding. Propionic and butyric acids were absent.

8. Petitjean. This site was sampled 5 times in a 41 day period of submergence, and consisted of one plot which received no organic matter supplement. The acids behaved in a step-wise manner, showing an initial rise in total concentration 7 to 13 days after flooding, after which there was an increase at a much slower rate followed by a rapid rise at the final sampling. In general this soil behaved in keeping with the fluctuations observed for the other sites.

9. Stansel. This was another plot site sampled during the 1963 season which did not receive organic matter. The soil was sampled 9 days after flooding and showed a continuous rise in acid concentration from this initial value to a peak of .05174 m.e. per 30 grams of water saturated soil at 18 days. The concentrations of total acids remained fairly high until the 58 day sampling, after which there was a rather rapid decline to .01902 m.e. per 30 grams of water saturated soil at the final sampling 79 days after flooding.

V. Fluctuations in Amounts of the Individual Acids

Acetic and propionic acids occurred commonly in all experimental sites throughout the 1963 rice season. Butyric acid was rarely present and then only

in minute quantities. In all soils acetic acid was the most abundant; its concentration values determining fluctuations in the compounded values for all acids. Butyric acid in general, was not evident in any of the sites until significant amounts of acetic and propionic acids appeared, thus butyric acid values were always associated with high values of the other two acids.

The appearance of the early and late season peak values as well as their corresponding lows varied from plot to plot. In some plots the pattern of fluctuation in acid concentrations was complete (Fig. 1). In others, where sampling was discontinued prematurely, the final decline was not detected (Fig. 2) and the last sampling showed high acid values.

VI. Results of Statistical Analysis of the 1963 Acid Data

Simple correlation coefficients were calculated to evaluate the significance of apparent relations between concentrations of propionic and acetic acids and between butyric and acetic acids. Replicate values for all sites were compounded from the data to provide a higher degree of reliability. In the case of acetic and propionic acids a correlation coefficient of +.824 was obtained, which proved to be highly significant at the 1 per cent level of probability. The coefficient for acetic and butyric acids was +.6277, which was also highly significant at the 1 per cent level of probability.

Regression coefficients were also calculated with acetic acid as the independent variable. The regression coefficient for acetic against propionic acid was +.3094. Each milliequivalent increase or decrease of acetic acid

concentration mediates a corresponding increase or decrease of .3094 milliequivalents in propionic acid. The mean regression equation calculated from these values was:

$$Y = .3094\ X - .00811,$$

where Y represents propionic acid and X acetic acid in milliequivalents.

The regression coefficient for acetic against butyric acid was .0203. Each milliequivalent increase or decrease in acetic acid (independent variable) produces a corresponding increase or decrease of .0203 milliequivalents in butyric acid (dependent variable). The regression equation in this case was:

$$Y' = .0203\ X + .000185.$$

These equations permit calculation of theoretical values for propionic and butyric acids corresponding to a given amount of acetic acid.

VII. Fatty Acid Content of Flood Water in Rice Fields

Samples of flood water taken at different intervals from three sites were analyzed for fatty acids in the same manner as the soil samples. The results show that acetic and propionic acids, and to a much lesser extent butyric acid were present in the water. Concentrations were approximately 1/15 of those of the corresponding soil sites and their fluctuations were similar to those in soil. However, concentration changes in water were not as abrupt as in the soil and the values throughout the season were more stable.

No attempt was made to ascertain the origin of acids in the flood water but the data indicate that they have diffused up from the soil below and may have

their origin in microbiological activity in submerged soil. Results from the individual sampling sites are presented (Table 9). The data indicate a correlation between soil acids and those found in the flood water but are insufficient for correlation analysis.

VIII. Relation Between Soil Depth and Fatty Acid Concentration in Rice Soils

Sampling at different depths was performed in some of the experimental sites to determine whether there was a depth gradient distribution of acids in the soil. A limited number of determinations of this kind was performed in the Wild, Compton, Fogelman, Davis and Petitjean sites (Tables 10, 11). Data on pH and Eh were also included.

It is of interest that the 0-1/2 inch soil layer of the Davis, Compton and Fogelman sites contained higher concentrations of total acids than the deeper layers; on the other hand, acids were more abundant in the 1/2-4.3 inch layer in the Wild and Petitjean sites than on the upper layer of soil, although the differences were slight.

Acetic acid was the predominant acid; propionic acid was more abundant in the upper layer of the Petitjean, Fogelman and Davis sites but was present in smaller quantities at the 0-1/2 inch level in the Compton and Wild plots. Butyric acid was either absent or present in trace amounts and then only in the upper layer of soil, except for the Davis soil in which it was more abundant at the 1/2 to 4.3 inch layer.

Although the number of samples was limited, the data indicate higher concentration of acids and a lower pH in the upper 0 to 1/2 inch layer of the submerged soils than at 1/2 to 4.3 inches.

IX. The Effect of Temperature on the Disappearance of Fatty Acids in Flooded Rice Soils

A laboratory experiment was designed to see if the decline of acids in rice fields could be related to temperature. Soils from the Wild and Richard plots were collected. The Wild soil was collected when it had already been submerged for 18 days, so as to represent a recently flooded soil; the Richard soil on the other hand, had been submerged 89 days when it was taken to the laboratory. These soils received a total of 16 m.e. of all acids, comprising 4 m.e. of formic, acetic, propionic and butyric acids, respectively. The jars containing the treatments were incubated at temperatures of 23°C and 30°C and there was a check without acids at each temperature (Tables 12, 13, 14, 15). The treatments were sampled at 9 days in the Wild soil and at 8 days in the Richard soil. Nematode counts showed no appreciable differences between the 2 temperatures. Analyses of acids showed a drastic reduction in the amounts of butyric and propionic acids at both temperatures and soils tested but acetic acid varied the least and was found in greater concentrations than the other acids. Formic acid disappeared from all soils at both temperatures. In general all of the acids were reduced in concentrations at both temperatures. Losses in general were greater at the higher temperature and there were no appreciable differences between Wild and Richard soils in the disappearance of acids.

X. The Effect of Corn Meal Additions on Soil Anaerobiosis

An experiment was conducted to determine the effect of a supplement of easily oxidizable organic matter to a submerged soil in the greenhouse. Four pots were filled with 14.37 Kgs. each of pasture soil from the Richard site. Two pots received no treatment and were used as checks; the other two pots received corn meal at 10 tons per acre. Nematode counts taken prior to flooding was used as a base level in interpreting results. The pots were flooded and then sampled at 3, 4, and 9 days in the same manner as the field sites. The samples were also analyzed for the same variables as in the field samples. The results are summarized in Tables 6, 7, 8, and Figures 7 and 8.

Acetic, propionic, and butyric acids were present. Propionic acid and particularly butyric acid were present in considerably larger amounts in the corn meal pots than in field samples or in check pots of this experiment. This is significant because it is these two acids that are most active against nematodes (28, 29). When the values of these acids were compared with number of nematodes throughout the 9 day sampling period (Fig. 7, 8), a pronounced inverse relationship emerged for the pots receiving corn meal. Nematodes increased in number in the check pots. Acids in the treated pots began to increase in an almost exponential fashion as early as 3 days after flooding and at all times they were higher than in the check pots. Butyric acid was present in the treated pots in larger amounts than propionic acid. This contrasts also with the data obtained from the check pots and field samples where propionic was more abundant than butyric acid. The rate of increase of acids in the corn meal

treatments was greatest in the first 4 days after flooding. All were at highest concentrations in the last sampling. The rate of acid production in the check pots was at all times considerably lower than in the treated pots.

Numbers of anaerobic spore forming bacteria increased in an exponential fashion in the corn meal pots (Table 8) and was correlated with the increase of fatty acids in these pots. The rate of growth of these bacteria in the check pots was not as pronounced as in the other treatments although their numbers in the first sampling was smaller in the corn meal treatments than in the checks.

The bacterial population in both the treated and nontreated pots was composed of essentially the same species, as listed.

1. Clostridium amylosaccharobutylpropylicum Beesch and Legg, 1947.
2. Cl. butylicum Donker, 1926
3. Cl. butyricum Prazmowski, 1880.
4. Cl. pasteurianum Winogradsky, 1895.

No attempt was made to determine what percentage of the bacterial population was composed of each of the species identified; however, the fact that butyric acid was the most abundant acid in the treated pots, suggests that Cl. butyricum could have been the most abundant bacterial species. This bacterium is known to be favored in its growth by the presence of corn meal (7). On the other hand, the presence of butyric acid cannot be solely ascribed to this bacterium since all other bacterial species identified in this experiment also produce it, although in smaller amounts. Cl. butylicum was isolated only from a nontreated pot.

These results explain the absence of large nemic populations in soils containing oxidizable forms of organic matter such as green manure or decomposing remains of vegetation. The quick killing of nematodes by propionic and butyric acids in high concentrations should be differentiated from the slow kill observed in rice fields. Since concentrations of these acids in rice fields are sublethal, their direct effects on nematodes are negligible or absent. Nematicidal action could be visualized only in special cases and limited to fields with high organic matter containing sufficient quantities of readily available components.

XI. The Anaerobic Spore Forming Bacteria in Submerged Rice Soils: Their Numbers, Isolation and Identification

A. Fluctuation in the size of the bacterial population.

The number of starch fermenting anaerobic spore forming bacteria in the 1963 experimental sites was determined throughout the rice season by their isolation to a modified Winogradsky medium high in dextrose and containing a potato infusion (3). The purpose of this work was to show a possible relationship in the soil between numbers of bacteria that would grow in such a medium and the fatty acids. Dilution tube counts were performed under anaerobic conditions from an aliquot of soil removed at each sampling.

The choice of a medium high in starch and dextrose for inoculation with spores of soil bacteria limited the bacterial counts to those species capable of metabolizing such materials and precluded the growth of other anaerobic spore forming bacteria found in soil. Carbon sources used in the medium were forms

of easily oxidizable organic matter that are known to be present in soil in small amounts. Large number of microbial species are capable of attacking such materials in well aerated soils (2, 77) but the degradation of sugars, starches, and similar materials in submerged soils proceeds at a slower rate and is limited to fewer bacterial species. The lower fatty acids are the common end products of this anaerobiosis; their kind and proportion varying in accordance with the anaerobic and facultative bacterial species present. The kind and amount of organic matter present in the soil prior to submergence is of the utmost importance in determining which species of bacteria shall predominate in the flooded environment.

Clostridium butyricum, a bacterium producing large amounts of butyric acid from starches and sugars had been found antibiotic against nematodes in flooded soils (28). It has also been found that propionic and butyric acids produced by this species were toxic to nematodes (29). These findings indicated the necessity of developing a method for counting this bacterium and related species which also produce these acids in the soil (47). In a preliminary greenhouse experiment (Tables 6, 7, 8, and Fig. 7, 8) four bacterial species were connected with the production of relatively high amounts of butyric, propionic and acetic acids, and the mixture of butyric and propionic acids was found inversely related to nematode numbers in the treatments supplemented with corn meal.

Bacterial counts from the 1963 field sites are in Table 4 and Figure 6. In general, the highest number of anaerobic spore forming bacteria isolated from

rice fields occurred during the first 10 days of submergence. This is in agreement with the nutritional requirements of these bacteria since their carbon sources are quickly exhausted in the soil. However, these bacteria are strict anaerobes and if their growth were arrested also by the presence of oxygen their numbers would be inversely related to concentrations of this gas. Oxygen was measured in the laboratory in experiments which closely duplicated conditions in rice fields with broadcast planting (Table 17). Levels of oxygen in these experiments were found comparable to those occurring in water held in air in the laboratory. Although no oxygen measurements were made in the field, it is evident that insofar as field conditions were realized, the decline of anaerobic bacteria could be related to the presence of oxygen in the rice field sites. Confirmation of this theory will necessitate oxygen measurements in the field.

B. The identity of bacterial isolates from soil samples taken from field sites during the 1963 rice season.

Bacterial species from the nine rice field sites in 1963 were:

1. Clostridium acetobutylicum McCoy et al, 1926.
2. Cl. amylosaccharobutylpropylicum Beesch and Legg, 1947.
3. Cl. butylicum Donker 1926.
4. Cl. butyricum Prazmowski, 1880.
5. Cl. pasteurianum Winogradsky, 1895.
6. Cl. roseum McCoy and McClung, 1935.

The first five species were present rather abundantly in all soils throughout the growing season, Cl. roseum on the other hand, was found only in the Davis

soil at the second sampling. Identity of some of the isolates was not established. The anaerobic bacterial flora in the sites can hardly be limited to 5 species or even to anaerobic spore formers. The use of a technique which was highly specific for anaerobic spore forming starch fermenting bacteria precluded the presence of other anaerobes which may have been common in these soils. In order to obtain a more complete picture of the bacterial soil flora at a particular time, different techniques and media would be required. The presence of these starch fermenting species serves as confirmatory evidence of the origin of the lower fatty acids. There may be other producers of such acids under submerged rice field conditions, particularly among the anaerobic nonspore forming bacteria.

XII. The Effect of Flooding on Nematodes in Louisiana Rice Fields

Plant parasites as well as total free-living nematodes were counted at all sampling dates from nine field sites during the 1963 rice season. Nematode numbers declined in general as time after flooding increased. In most cases, however, the decline in numbers, illustrated by data from the Richard or Caffey (B) plots was slow from the beginning and continued throughout the entire rice season (Fig. 3, 4, 5).

Increase of nematode numbers was observed in some soils at 10-15 days after flooding; this increase coincided with the application of fertilizer. Increase in the number of rice roots would result in an increase of the feeding area for nematodes.

Fluctuation in numbers of plant parasitic nematodes in general followed trends for total nematodes although variations in numbers of plant parasites were more erratic.

XIII. Statistical Analysis of the 1963 Nematode Field Data

A correlation coefficient of -.46 was obtained by relating nematode numbers to days after flooding. This coefficient was highly significant at the 1 per cent level of probability indicated a definite association between the two variables.

Comparisons were made between the percentage decrease in numbers of plant parasites and total nematodes (Table 5). A more rapid decline of plant parasites occurred when the highest number of nematodes in the first two samplings of each soil was used as a base line. The slower decline of total nematodes is believed due to the high proportion of saprozoics and their higher apparent resistance to toxic substances (22, 23).

XIV. Variability in Populations of Individual Species of Nematodes During the 1963 Rice Season

Species of plant parasitic nematodes were determined in all samplings in order to learn more about fluctuations in their numbers. Populations of T. martini, R. oryzae, Helicotylenchus erythrinae (Zimmermann, 1904) Golden 1956, Criconemoides sp., and Pratylenchus zeae Graham 1951, were encountered. T. martini and R. oryzae were present commonly throughout the season, whereas

the other 3 species disappeared early in the season, usually before the third sampling. Populations of species of Criconemoides recovered quickly after drainage of the soil in rice fields, suggesting that the eggs persisted through the flooding period. The fact that this nematode was abundant in rice soils of pasture phases in three-year rotations support the rice field data. Spiral and lesion nematode populations recovered also after drainage of fields, but not to the same degree as Criconemoides sp. T. martini and R. oryzae were found commonly in the rice area in both flooded and drained soils.

XV. Laboratory Assay of Fatty Acids and Sulfides Against Nematodes

The slow decline of nematode populations in rice fields and the pattern of fatty acid concentrations at uniformly low levels eliminated for the most part any possibility of direct antibiotic effects of acids against nematodes under Louisiana rice field conditions. This was confirmed by in vitro tests, in which water solutions of the acids at concentrations comparable to those found in the field and adjusted to pH levels equivalent to those in rice soils, were used against swarming populations of T. martini. A number of such tests conducted under both aerobic and anaerobic conditions demonstrated that rice field acids had no effect on the nematodes.

The presence of a black layer near the surface of submerged rice soil was observed in laboratory experiments. This layer appeared commonly after 2 weeks incubation and was followed closely by an iridescent, rust-colored

pellicle on water and soil surfaces. Analyses of the black layer and of the pellicle showed that the former was very rich in metal sulfides while the latter was composed primarily of oxides of iron. The levels and types of acids maintained in rice fields throughout the growing season, the characteristic slow decline of nematode populations in rice fields, the failure of acids in field mixtures and concentrations to kill nematodes in vitro, coupled with observations on transformations of sulfides and iron in rice soils suggested that hydrogen sulfide might be the long sought antibiotic factor. The presence of hydrogen sulfide in rice fields had been reported by several workers (48); in some cases in connection with physiological diseases of rice (44).

Preliminary tests with water solutions of hydrogen sulfide ranging from 27 to 314 ppm, in which swarms of T. martini were incubated in sealed vials for varying periods of time, resulted in death of the nematodes. Time of exposure to the solution was an important factor. A further test was conducted with water solutions of hydrogen sulfide at concentrations of 62.7, 32.7, 16.4 and 9.3 ppm. Swarms of T. martini were put in 5 ml vials containing the solutions and capped; check vials contained distilled water. Nematodes were observed for mobility at different intervals of time. Results in terms of time required for 100 per cent kill are in Figure 9.

As a further step in these laboratory trials, the action of fatty acids at field concentrations on ferrous sulfide was studied. A test was prepared with one series containing mixtures of acetic, propionic and butyric acids at concentrations of 196, 36, and 8 ppm, respectively. A second series was

prepared in which finely-ground ferrous sulfide was added in excess to these acid solutions. Finally each series was divided into two treatments and their pH levels were adjusted to 3.5 and 7.0 with HCl and NaOH. Each treatment was replicated in 8 vials. Vials were inoculated each with 3-4 thousand individuals in swarms of T. martini. Observations at 86 hours showed that nematodes were killed by the ferrous sulfide treatment at pH 3.5. Twelve days exposure resulted in the death of nematodes in the acid treatment at pH 3.5 but not at pH 7.0.

An additional test of ferrous sulfide at pH levels ranging from 3.4 to 6.4 in citrate-phosphate buffer (9) and incubated under both aerobic and anaerobic conditions showed that ferrous sulfide was toxic to T. martini at all pH levels under anaerobic conditions and at pH 3.5 under aerobic conditions.

XVI. Determination of Hydrogen Sulfide in Submerged Rice Soils in 1964

Sulfides are known to result from the activities of Desulfovibrio sp. and some of the anaerobic heterotrophic bacteria decomposing organic sulfur in organic matter. Hydrogen sulfide can result from the direct action of Desulfovibrio sp. on sulfates or from the interaction of fatty acids with metal sulfides (40). Hydrogen sulfide determinations were made on soil samples removed from 3 field sites during the 1964 rice season (Fig. 10). There were constant increases in hydrogen sulfide beginning 5-7 days after flooding in the 3 plots. Levels of hydrogen sulfide ranged from 10 to 40 ppm at the end of the season, at 136

days after flooding. Nematode numbers were inversely proportional to concentrations of hydrogen sulfide.

XVII. The Relationship of Oxygen to the Decline of Nematode Populations in Rice Fields

The earliest explanation for the decrease of nematode populations in submerged soils was drowning of the nemas caused by lack of oxygen (8, 13, 24). This was generally accepted but never proved. The laboratory experiments of Van Gundy, Stolzy and coworkers (58, 74, 75), subjecting nematodes in soil to different levels of oxygen, lended support to this explanation. Hollis and Johnston and Johnston (19, 26, 28) connected the decline of nematodes in rice fields with antibiotic factors, thus throwing doubt on the oxygen depletion theory. Experiments were run in the present work to determine level of oxygen, both in vitro and in flooded rice soil, and its role if any, in maintaining viability of T. martini in vitro. Atmospheres of carbon dioxide, nitrogen, and hydrogen were established over nematode suspensions and the absence of oxygen confirmed periodically by means of chronoamperometric measurements with an oscilloscope and platinum electrodes inserted in suspensions in the flasks. Oxygen was shown to be absent throughout these tests. The flasks were opened after 14 days and the nematodes were observed for viability. Those subjected to a carbon dioxide atmosphere were found inmobilized but later they recovered and showed a mortality of about 20 per cent. Nematodes in the nitrogen and hydrogen treatments were unaffected. These results were at variance with those of Van Gundy et al (74). However, it was recognized that effective evaluation of the

role of oxygen in flooded rice soils would require assessment of oxygen at different depths in flooded rice soils over a period of time.

The effects of rice roots on oxidation-reduction potentials has been reported (4, 48), and connected with the ability of rice plants to transport and to release oxygen to the roots (50, 76). Oxygen in rice soils should result in a depressive effect on the anaerobic soil microflora responsible for the formation of the organic acids and sulfides active against nematodes. This could thus account in part for the presence of reduced populations of nematodes in rice soils containing lethal concentrations of hydrogen sulfide.

A cellulose nitrate cylinder 24 x 5.5 inches was packed with Wild soil from the 1963 plot, flooded, planted to Bluebonnet-50 rice after drainage then reflooded. Pairs of platinum microelectrodes were inserted laterally in the cylinder at depths of 0, 3, 5, 9.5, and 15 cms from the soil upper surface. Oxygen was measured chronoamperometrically by utilizing an oscilloscope, in accordance with methods described by Lemon, Lingane and Willey (33, 34, 80). Readings were taken for a period of 50 days following submergence and were accompanied by check readings taken in air-saturated water and in water which had been swept with nitrogen for a sufficiently long period to insure removal of oxygen. All readings were taken at a room temperature of 27°C.

The results (Table 17) show that conditions in a flooded rice soil are not anaerobic; oxygen is present at all depths to which the root system extends. Relatively high oxygen levels (Approximately 6 to 12 ppm) were found in

submerged soil around rice roots at all depths tested throughout the experiment. Oxygen levels at any given depth decreased slightly with time of submergence. The presence of oxygen in rice soils is directly related to quantity of rice roots in the soil; rice fields planted in a broadcast fashion could be expected to contain higher levels of oxygen earlier in the growing season than fields where rice was drilled.

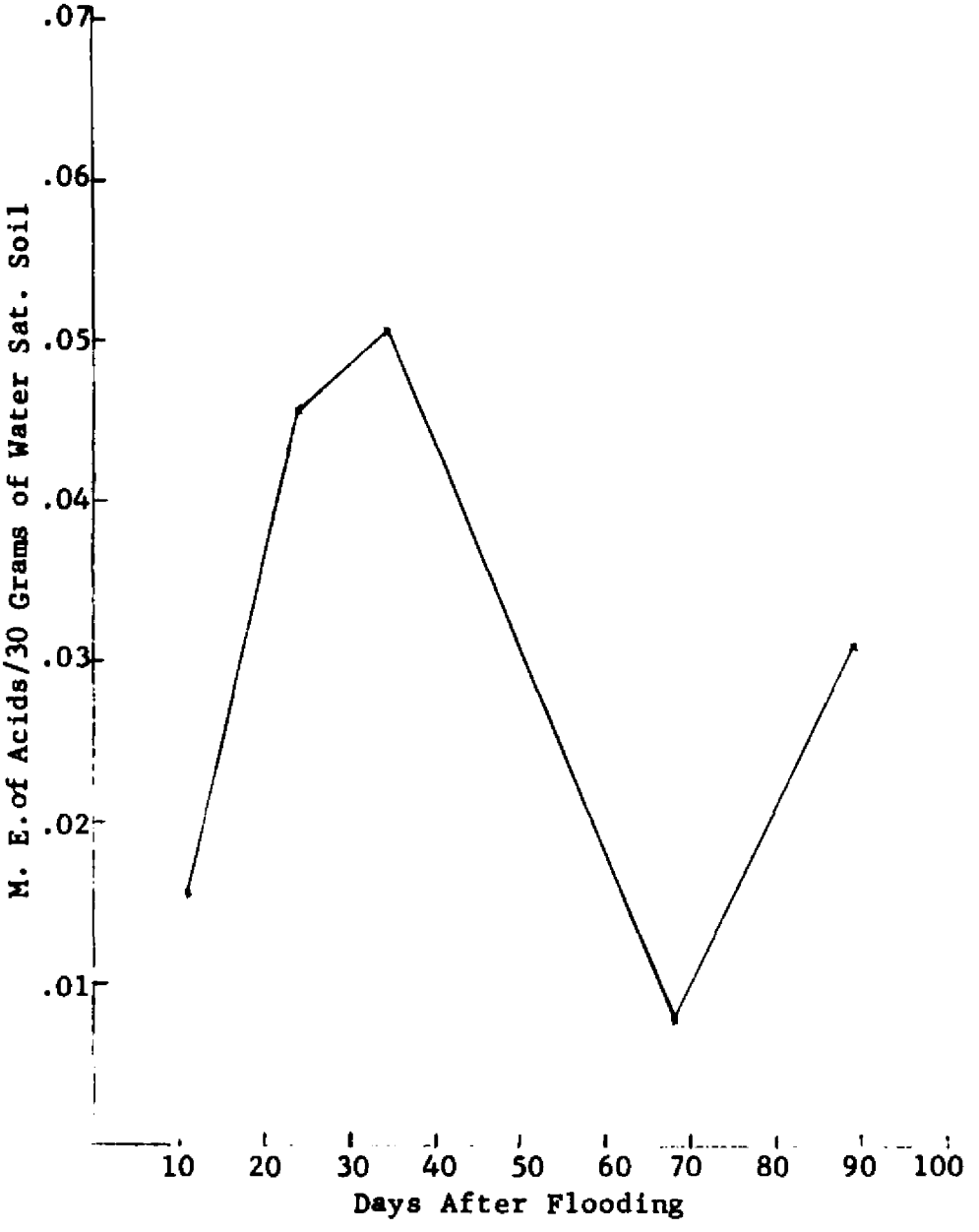

Figure 1. Fluctuation pattern in concentrations of total fatty acids (Richard B plot 1963 rice season).

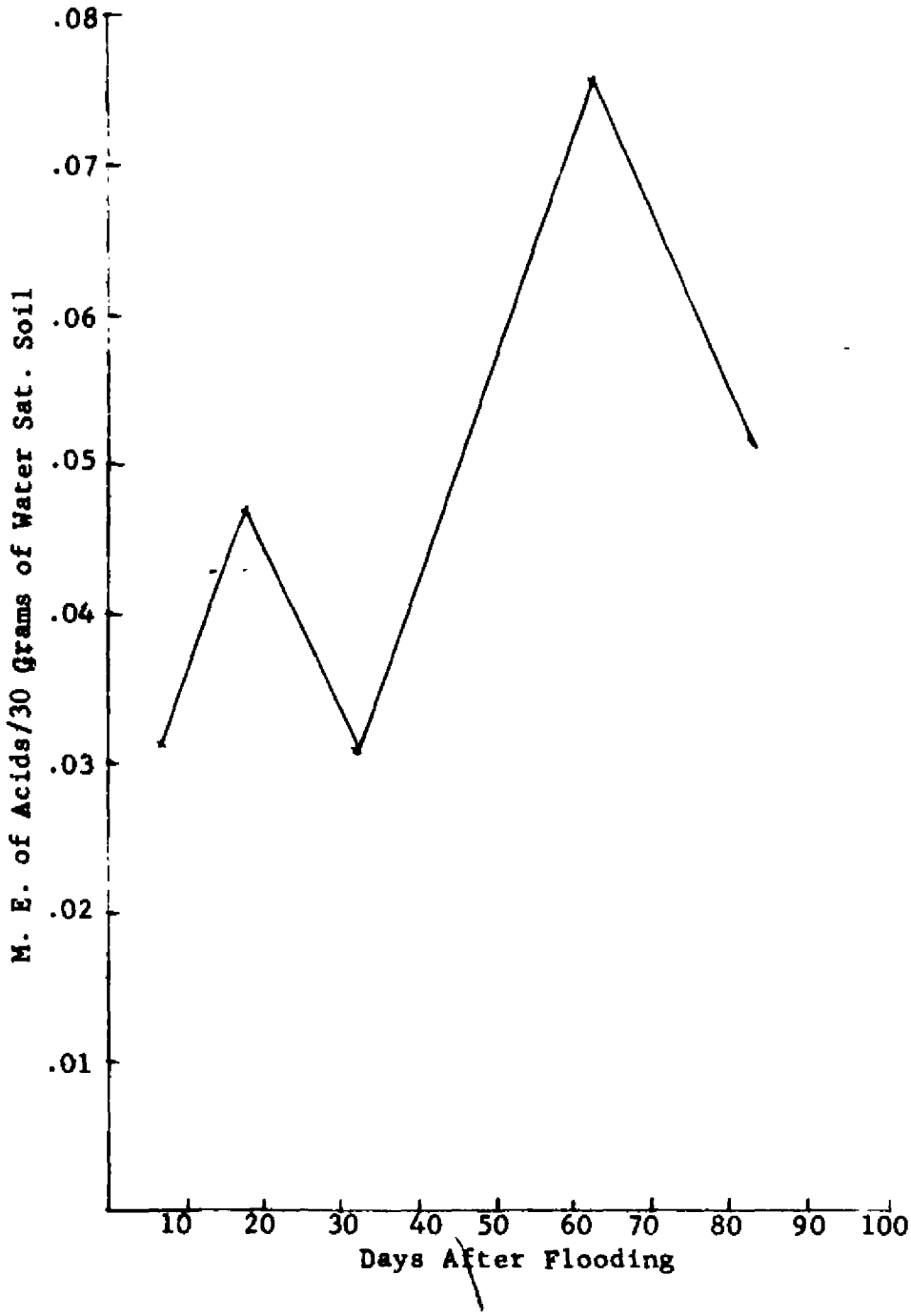

Figure 2. Fluctuation pattern in concentrations of total fatty acids (Caffey A plot 1963 rice season).

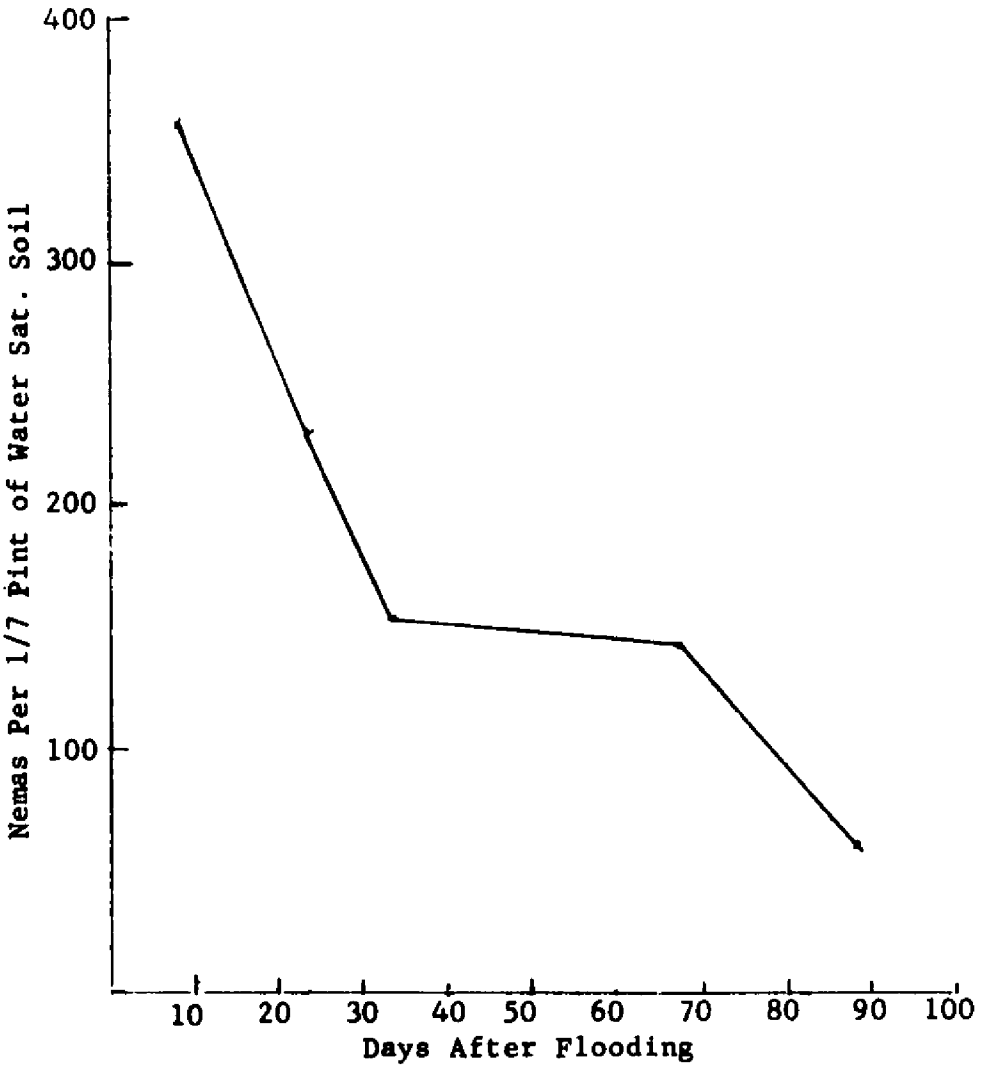

Figure 3. Trend of total nematode population (Richard A plot 1963 rice season).

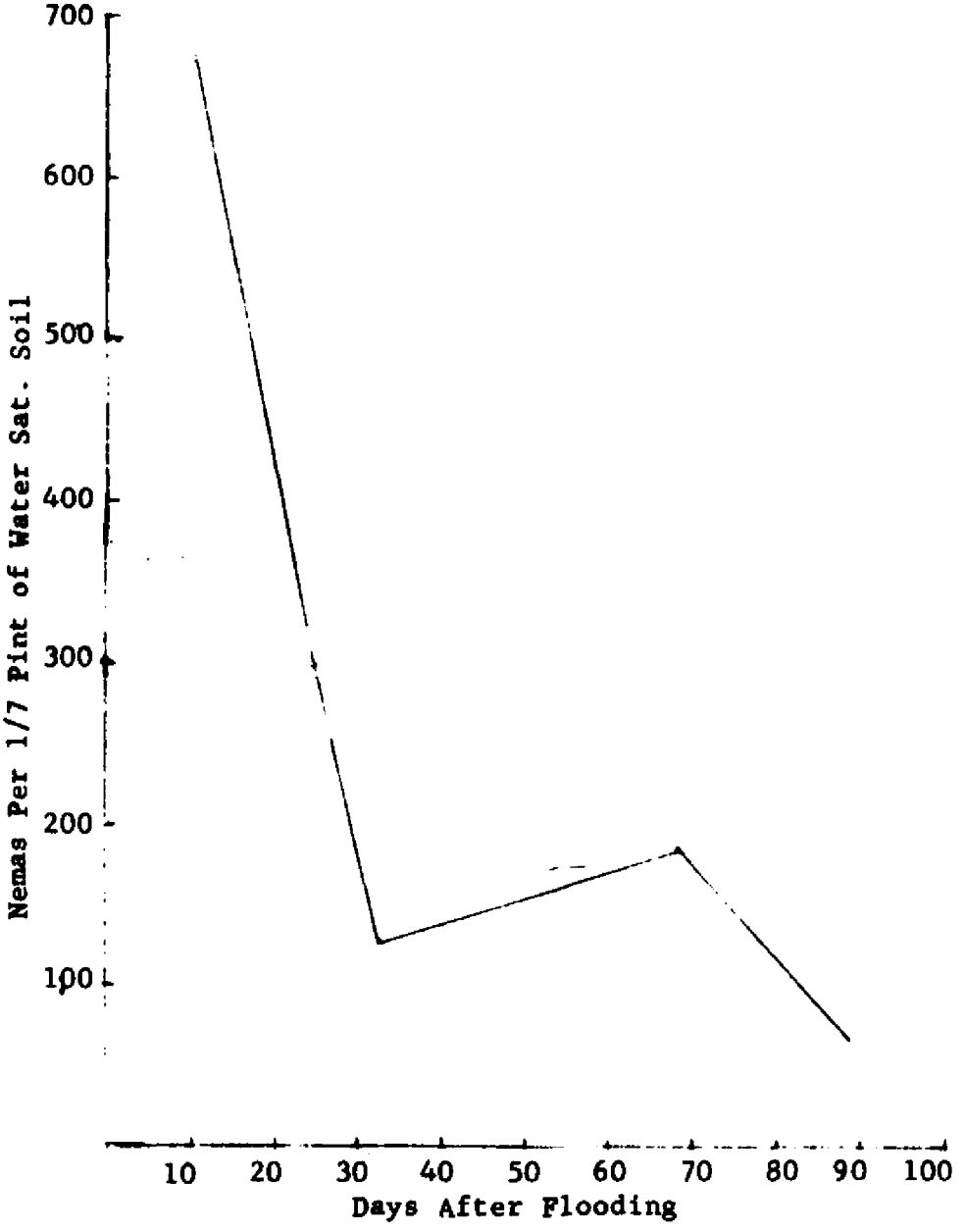

Figure 4. Trend of total nematode population (Richard B plot 1963 rice season).

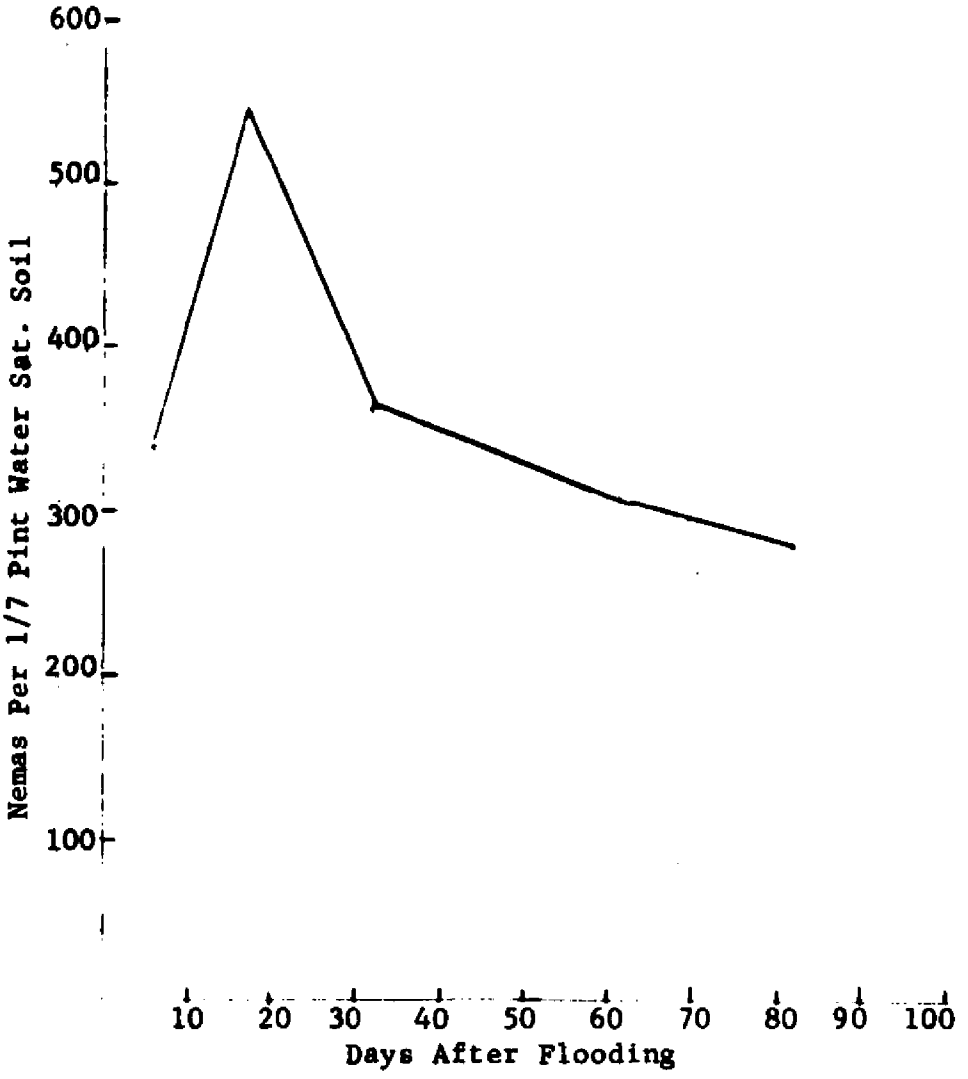

Figure 5. Trend of total nematode population (Caffey B plot 1963 rice season).

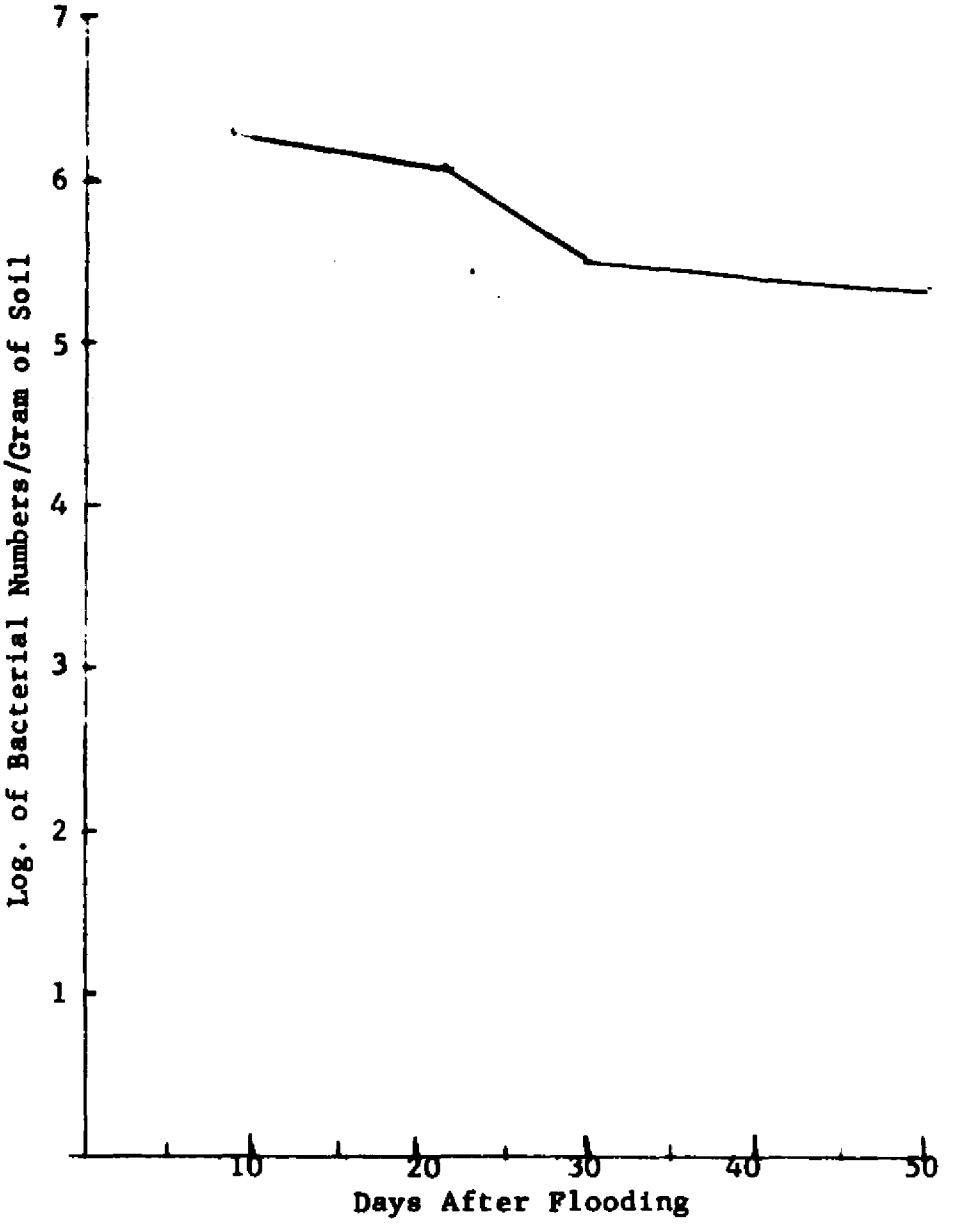

Figure 6. Population trend of anaerobic spore-forming bacteria (Compton B plot 1963 rice season).

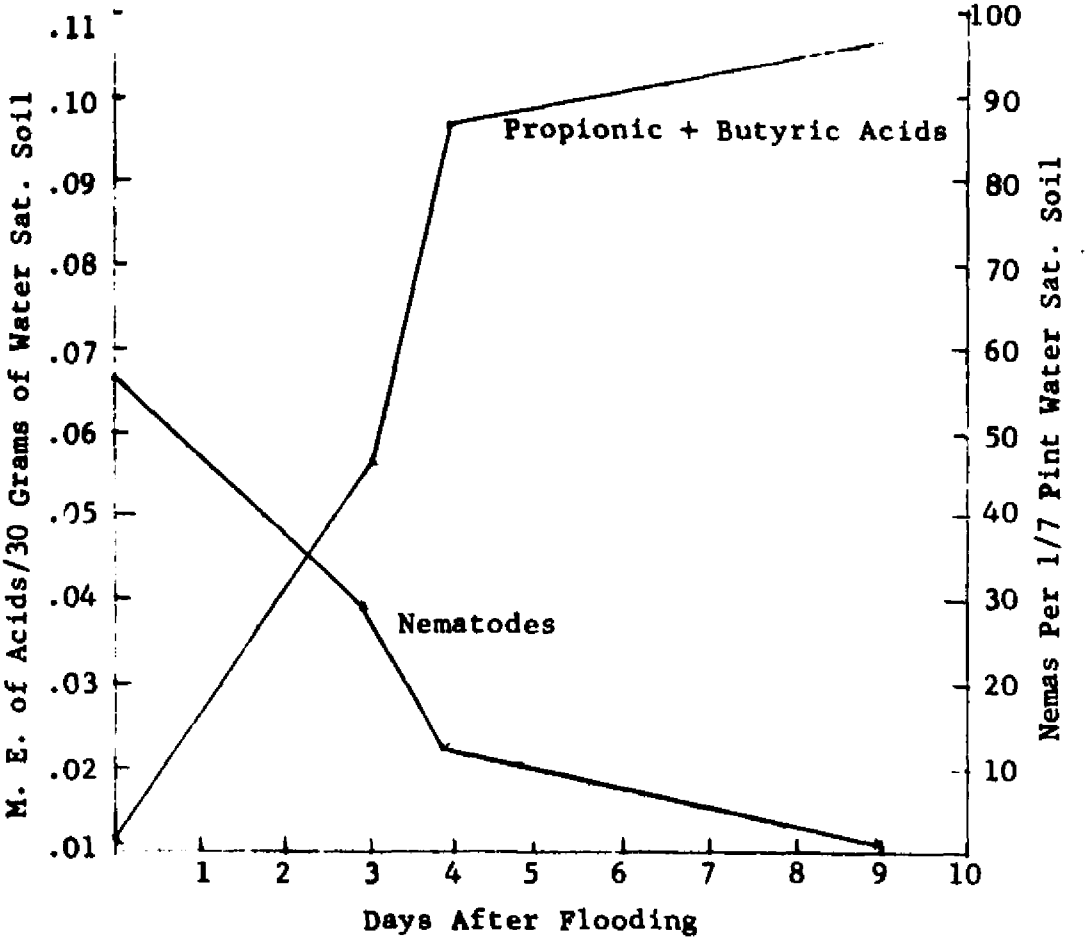

Figure 7. Decline of total nematode population in Richard soil supplemented with cornmeal and its relation to concentrations of propionic + butyric acids in greenhouse pots..

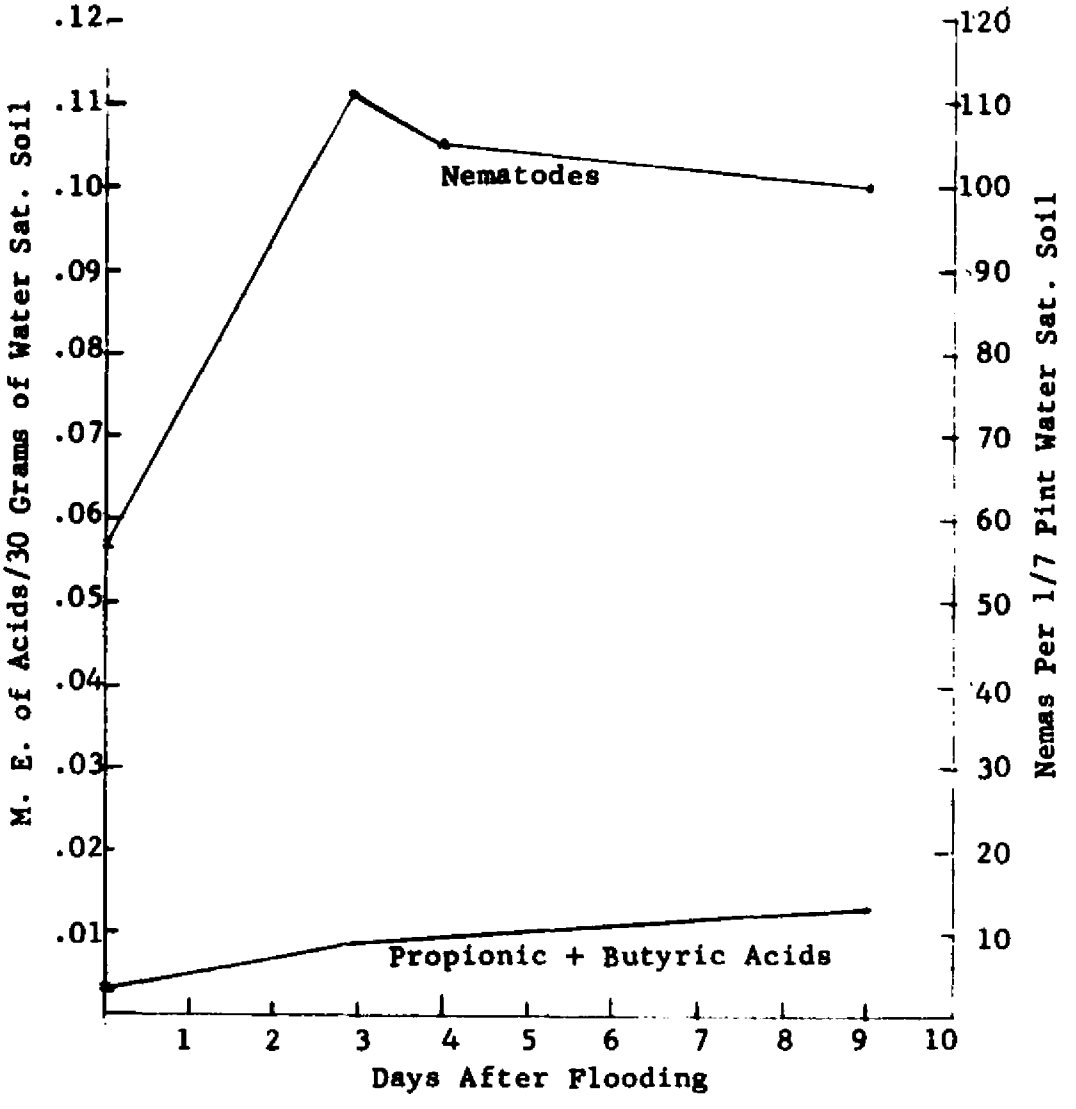

Figure 8. Increase of total nematode population in the presence of field concentrations of propionic + butyric acids in Richard check soil in greenhouse pots.

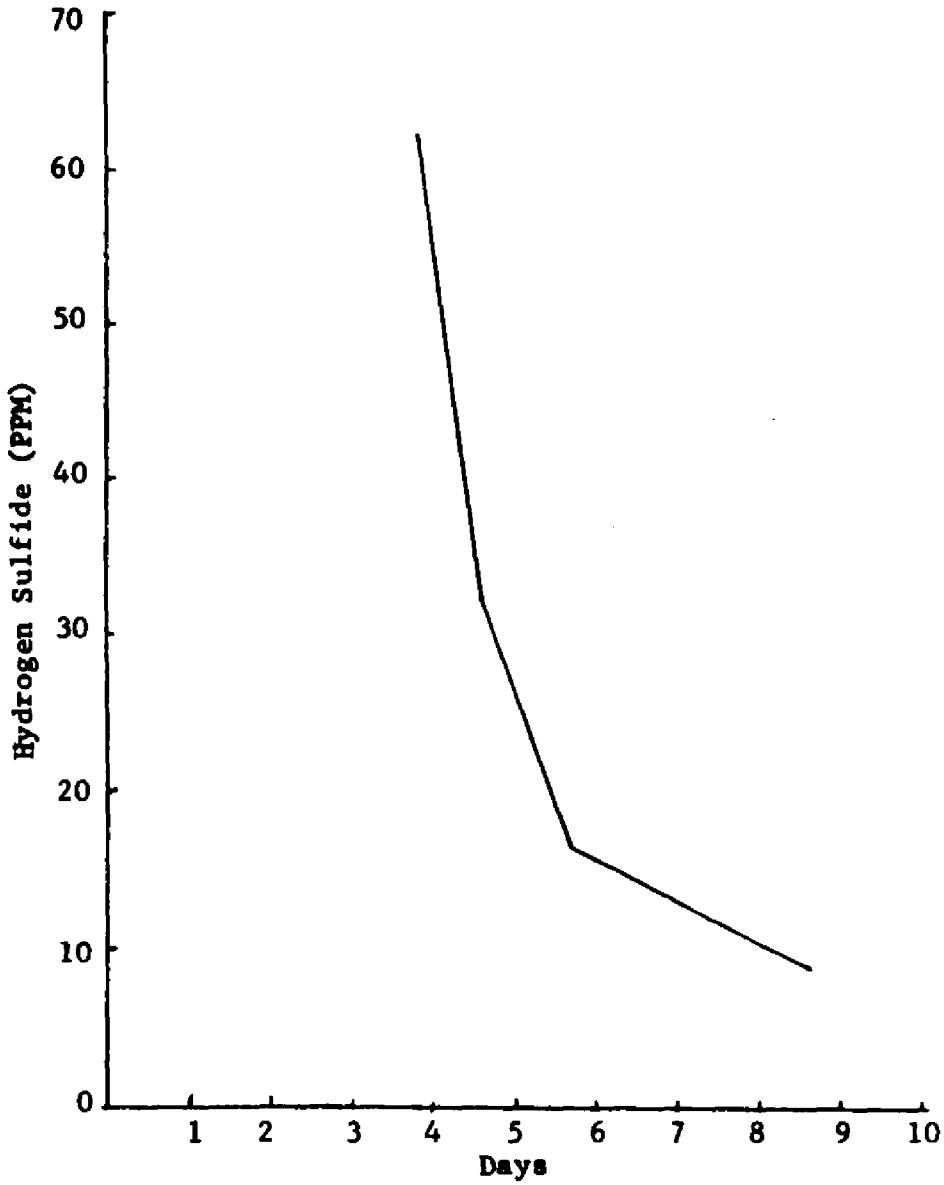

Figure 9. Relation between hydrogen sulfide concentration and time required for 100 per cent kill of swarming populations of T. martini in sealed vials.

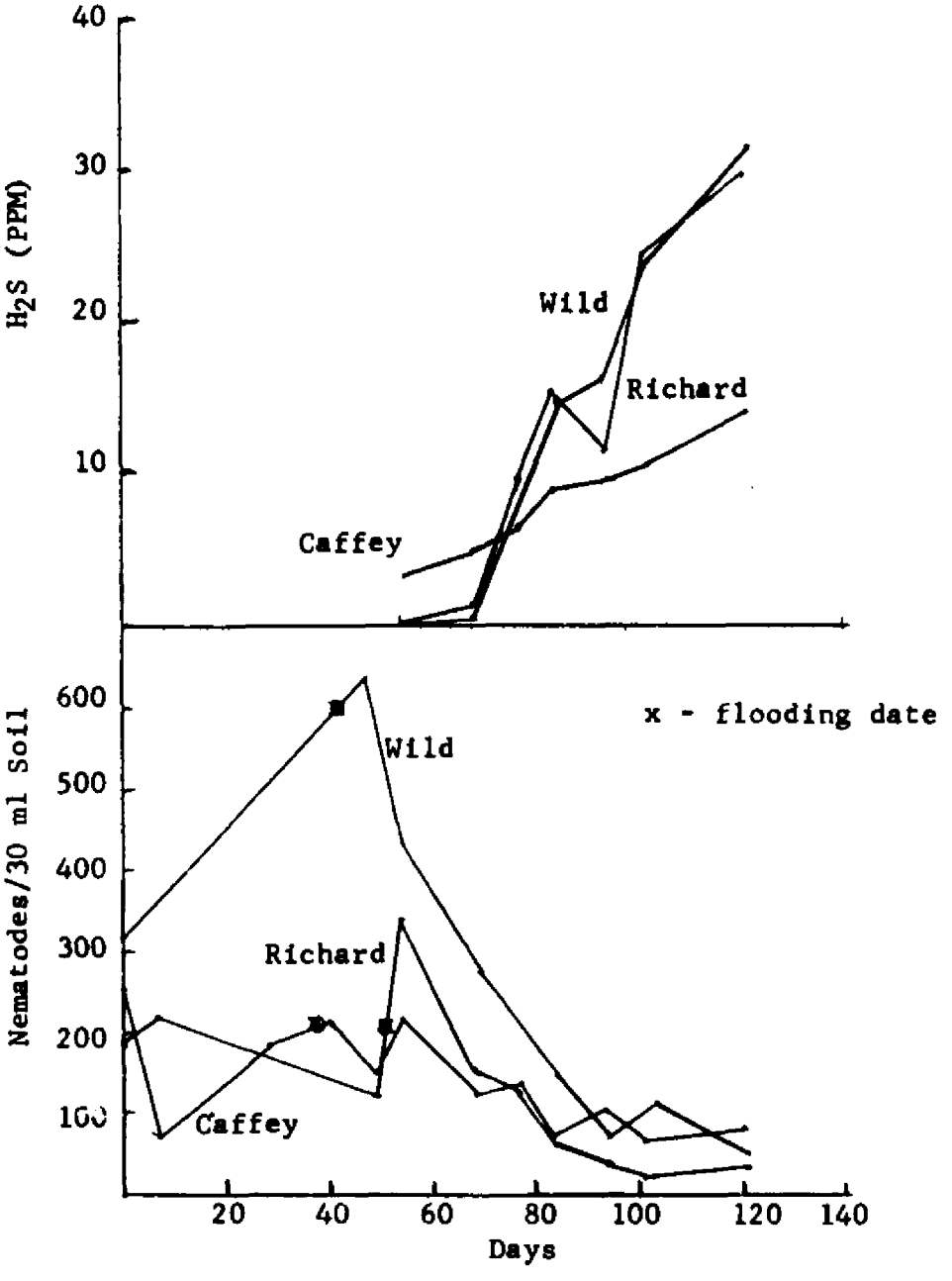

Figure 10. Total nematode populations in 3 rice fields in 1964 and hydrogen sulfide concentrations in the soil water phase. First traces of H_2S appeared in Wild and Richard plots 5-7 days after flooding.

Figure 11. Standard curves for H_2S in normal NaOH at pH 12 and a temperature of 25°C. Diffusion current at 0.84 volt (versus standard calomel electrode) is proportional to HS^- concentration. Steps in curves, denoted by arrows, represent H_2S concentrations 13.43, 6.71, 3.35, 1.67 ppm.

Table 2. Physical and chemical variables in soil anaerobiosis, determined on soil samples from flooded rice fields in 1963.

Sampling Site	Sampling (days after flooding)	pH	Eh (millivolts)	Per cent Carbon	Per cent Nitrogen
Petitjean	7	6.60	+ 86	1.11	.1470
	13	6.65	+ 81	0.99	.1424
	20	6.80	- 36	1.00	.1558
	34	6.85	- 93	1.07	.1641
	41	6.94	-145	0.84	.1457
	48	--	--	0.79	--
Caffey (A plot)[1]	7	6.30	+117	0.67	.1156
	18	6.00	- 59	0.87	.1121
	32	6.50	- 14	0.93	.1341
	62	6.70	- 49	0.90	.1122
	82	6.65	+ 11	0.85	.1229
Caffey (B plot)	7	6.40	+183	0.62	.1175
	18	6.10	-	0.71	.1281
	32	6.50	+ 91	0.73	--
	62	6.80	- 26	0.71	.1122
	82	6.90	- 4	0.74	.1132
Wild (A plot)	8	6.60	+ 16	1.20	.1396
	15	6.68	- 4	0.98	.1366
	22	6.40	- 44	0.92	.1305
	36	6.33	- 86	1.33	.1358
Wild (B plot)	8	6.50	+111	1.01	.1372
	15	6.77	- 24	1.13	.1438
	22	6.45	- 14	0.77	.1229
	36	6.78	- 53	1.01	.1327
Fogelman (A plot)	2	6.00	+ 96	0.95	.1279
	9	6.65	- 49	0.99	--
	16	6.85	- 32	0.92	.1215
Fogelman (B plot)	2	6.50	+ 11	1.28	.1493
	9	6.60	+136	0.96	.1234
	16	6.87	- 22	0.62	.1171

Table 2. Continued

Sampling Site	Sampling (days after flooding)	pH	Eh (millivolts)	Per cent Carbon	Per cent Nitrogen
Stansel	9	5.20	+341	5.98	.2497
	18	5.30	+176	2.09	.2455
	31	5.60	+146	2.58	.3507
	58	5.40	+171	2.39	.2831
	79	5.70	- 58	2.46	.2630
Byler (A plot)	59	6.60	- 39	0.74	.1070
	72	6.57	- 46	0.71	.0961
Byler (B plot)	59	6.20	- 24	0.79	.0943
	72	6.50	- 24	0.82	.1010
Compton (A plot)	9	6.25	- 22	0.68	.1094
	22	6.35	- 74	0.79	.1253
	30	6.50	- 54	0.85	.1157
	50	6.40	+ 96	0.94	.1055
	71	6.38	- 47	0.76	.0982
	85	--	--	--	--
Compton (B plot)	9	6.20	- 52	0.55	.1232
	22	5.95	+ 91	0.76	.1164
	30	6.40	- 94	0.93	.1042
	50	6.60	+ 6	0.78	.1112
	71	6.36	- 50	0.56	.1044
Davis (A plot)	10	5.60	+269	0.83	.1024
	18	6.50	-109	0.72	.1066
	32	6.35	- 54	0.75	.0989
	52	6.35	+ 16	0.84	.1028
Davis (B plot)	10	6.00	+ 8	0.74	.1192
	18	6.40	+ 46	0.81	.1143
	32	6.45	- 54	0.84	.1101
	52	6.30	- 29	0.77	.0887

Table 2. Continued

Sampling Site	Sampling (days after flooding)	pH	Eh (millivolts)	Per cent Carbon	Per cent Nitrogen
Richard (A plot)	11	6.82	- 32	0.97	.1074
	25	6.95	- 54	0.99	.1002
	34	6.75	- 67	0.74	.0944
	69	6.55	- 94	0.72	.0912
	89	6.60	- 34	0.56	.0851
Richard (B plot)	11	6.55	- 22	0.71	.0996
	25	6.90	- 79	1.05	.0961
	34	6.78	- 39	0.73	.0874
	69	7.00	-134	0.66	.0832
	89	6.45	+ 91	0.56	.0706

[1]A plot supplemented with 8.6 tons of cotton seed hulls per acre, B plot, no organic matter supplement.

Table 3. Fatty acids in soil anaerobiosis, determined on soil samples from flooded rice fields in 1963.

Sampling Site	Sampling (days after flooding)	M.E. Fatty Acids[1]			
		Total	Acetic	Propionic	Butyric
Petitjean	7	.00758	.00758	.00000	.00000
	13	.02612	.02482	.00140	.00000
	20	.02826	.02620	.00140	.00066
	34	.03174	.03000	.00174	.00000
	41	.04864	.04482	.00278	.00104
Caffey (A plot)[2]	7	.03164	.02976	.00138	.00050
	18	.04796	.04560	.00236	.00000
	32	.04796	.04560	.00236	.00000
	62	.07618	.07618	.00000	.00000
	82	.05258	.05098	.00160	.00000
Caffey (B plot)	7	.02884	.02756	.00128	.00000
	18	.01826	.01708	.00118	.00000
	32	.03100	.02854	.00246	.00000
	62	.02624	.02464	.00160	.00000
	82	.12338	.11362	.00540	.00436
Wild (A plot)	8	.03592	.03258	.00184	.00150
	15	.04004	.03792	.00126	.00086
	22	.03756	.03216	.00426	.00120
	36	.10880	.10192	.00526	.00162
	43	.03872	.03488	.00384	.00000
Wild (B plot)	8	.03560	.03452	.00108	.00000
	15	.03670	.03520	.00150	.00000
	22	.02889	.02662	.00227	.00000
	36	.02672	.02514	.00100	.00058
	43	.04180	.03712	.00344	.00124
Fogelman (A plot)	2	.00372	.00372	.00000	.00000
	9	.04058	.03864	.00142	.00052
	16	.03256	.03256	.00000	.00000
Fogelman (B plot)	2	.00272	.00272	.00000	.00000
	9	.04328	.04204	.00124	.00000
	16	.02902	.02814	.00088	.00000

Table 3. Continued

Sampling Site	Sampling (days after flooding)	M.E. Fatty Acids[1] Total	Acetic	Propionic	Butyric
Stansel	9	.02196	.02066	.00130	.00000
	18	.05174	.04934	.00240	.00000
	31	.04772	.04500	.00186	.00076
	58	.03982	.03834	.00148	.00000
	79	.01902	.01768	.00074	.00060
Byler (A plot)	59	.00372	.00372	.00000	.00000
	72	.00406	.00406	.00000	.00000
Byler (B plot)	59	.01152	.01152	.00000	.00000
	72	.01018	.01018	.00000	.00000
Compton (A plot)	9	.07060	.06100	.00828	.00132
	22	.05162	.04966	.00156	.00040
	30	.05100	.04660	.00362	.00072
	50	.04048	.03766	.00190	.00092
	71	.02745	.02734	.00008	.00003
Compton	9	.05032	.04766	.00224	.00092
	22	.04090	.03900	.00190	.00000
	30	.00666	.00666	.00000	.00000
	50	.04744	.04466	.00172	.00106
	71	.03058	.02800	.00192	.00066
Davis (A plot)	10	.03664	.03564	.00100	.00000
	18	.10956	.08036	.02716	.00204
	32	.01212	.01128	.00084	.00000
	52	.03156	.02800	.00200	.00156
Davis (B plot)	10	.05616	.05418	.00150	.00048
	18	.06484	.06038	.00350	.00096
	32	.01146	.00946	.00116	.00084
	52	.02540	.02540	.00000	.00000
Richard (A plot)	11	.02583	.02292	.00228	.00063
	25	.03470	.03244	.00224	.00102
	69	.01838	.01610	.00228	.00000
	89	.04542	.04146	.00318	.00078

Table 3. Continued

Sampling Site	Sampling (days after flooding)	M.E. Fatty Acids[1]			
		Total	Acetic	Propionic	Butyric
Richard (B plot)	11	.01650	.01560	.00090	.00000
	25	.04636	.02898	.01612	.00126
	34	.05038	.04830	.00160	.00048
	69	.00820	.00732	.00090	.00000
	89	.03138	.03024	.00114	.00000

[1]Values are milliequivalents per 30 grams of water saturated soil.

[2]A plot was supplemented with 8.6 tons of cotton seed hulls per acre; the B plot received no organic matter supplement.

Table 4. Nematodes and bacteria in soil anaerobiosis, determined on soil samples from flooded rice fields in 1963.

Sampling Site	Sampling (days after flooding)	Number of bacteria per gm of soil	Number of nemas per 1/7 pint of soil	Number of Plant Parasitic Nemas per 1/7 pint of soil
Petitjean	7	2.57×10^6	72	24
	13	3.42×10^5	100	24
	20	1.13×10^5	80	10
	34	--	12	0
	41	--	68	13
	48	--	43	8
Caffey (Plot A)[1]	7	2.57×10^6	520	2
	18	2.28×10^6	760	12
	32	2.57×10^6	215	4
	62	3.42×10^5	305	8
	82	1.32×10^6	169	8
Caffey (Plot B)	7	1.32×10^7	340	5
	18	2.43×10^5	540	12
	32	2.57×10^6	360	2
	62	1.32×10^6	300	25
	82	2.28×10^6	234	9
Wild (Plot A)	8	2.57×10^6	250	9
	15	2.28×10^6	680	24
	22	1.32×10^6	208	10
	36	--	80	7
	43	--	105	33
Wild (Plot B)	8	1.32×10^6	340	28
	15	5.00×10^5	400	4
	22	1.86×10^5	230	20
	36	--	100	5
	43	--	93	7
Fogelman (Plot A)	2	1.32×10^7	500	165
	9	7.72×10^5	400	22
	16	1.32×10^6	210	16
	44	--	29	9

Table 4. Continued

Sampling Site	Sampling (days after flooding)	Number of bacteria per gm of soil	Number of nemas per 1/7 pint of soil	Number of Plant Parasitic Nemas per 1/7 pint of soil
Fogelman	2	2.57×10^7	96	6
(Plot B)	9	2.57×10^6	110	20
	16	1.86×10^5	24	2
	44	--	54	5
Stansel	9	2.28×10^7	450	325
	18	2.57×10^6	100	84
	31	2.57×10^6	380	280
	58	2.57×10^6	195	121
	79	-	5	2
Byler	59	2.28×10^6	64	2
(Plot A)	72	7.72×10^5	64	0
Byler	59	2.57×10^6	130	2
(Plot B)	72	7.72×10^5	200	4
Compton	9	2.28×10^6	740	460
(Plot A)	22	2.28×10^6	460	100
	30	3.42×10^5	340	150
	50	3.42×10^5	300	104
	71	--	282	149
	85	--	102	50
Compton	9	2.28×10^6	500	375
(Plot B)	22	1.32×10^6	160	60
	30	5.00×10^5	200	68
	50	3.42×10^5	240	120
	71	--	59	24
	85	--	51	5
Davis	10	2.28×10^7	1760	346
(Plot A)	18	2.28×10^6	240	68
	32	2.28×10^7	240	10
	52	5.00×10^5	258	8
	73	--	175	29

Table 4. Continued

Sampling Site	Sampling (days after flooding)	Number of bacteria per gm of soil	Number of nemas per 1/7 pint of soil	Number of Plant Parasitic Nemas per 1/7 pint of soil
Davis	10	2.57×10^6	740	88
(Plot B)	18	2.28×10^6	540	60
	32	1.32×10^6	528	108
	52	2.57×10^6	88	10
	73	--	118	40
Richard	11	2.28×10^7	360	34
(Plot A)	25	2.28×10^6	229	23
	34	--	155	16
	69	2.57×10^6	148	24
	89	7.00×10^4	78	6
Richard	11	3.14×10^5	680	92
(Plot B)	25	5.00×10^5	300	55
	34	2.57×10^6	120	16
	69	1.32×10^6	188	12
	89	2.57×10^3	74	8

[1]Plots A received 8.6 tons of cotton seed hulls per acre, plots B received no supplement.

Table 5. Per cent decline of nematode populations as a result of flooding of rice fields in 1963.

Sampling Site	Sampling (days after flooding)	Per cent decrease of all nemas	Per cent decrease of plant parasitic nemas	Difference (plant parasites--all nemas)
Fogelman	2	0	0	0
	9	15	76	51
	16	61	90	29
	44	86	92	6
Davis	10	0	0	0
	18	69	70	1
	32	69	73	4
	52	86	96	10
	73	89	84	- 5
Richard	11	0	0	0
	25	49	38	-11
	34	74	75	1
	69	68	71	3
	89	85	89	4
Compton	9	0	0	0
	22	50	81	31
	30	56	74	18
	50	56	73	6
	71	73	79	6
	85	88	94	0
Stansel	9	0	0	0
	18	78	74	- 4
	31	16	14	- 2
	58	57	63	6
	79	99	99	0
Petitjean	13	0	0	0
	20	20	58	38
	34	88	100	12
	41	32	46	4
	48	57	66	9

Table 6. Effect of corn meal on related variables in Richard soil submerged under water in the greenhouse.

Treatment	Sampling (days after flooding)	pH	Eh (millivolts)	Per cent Carbon	Per cent Nitrogen
Soil +	0	4.90	+526	0.52	.0488
Corn Meal	3	4.96	-179	0.58	.0568
(Pot I)	4	4.70	- 54	0.59	.0544
	9	4.50	+ 31	0.59	.0607
Soil +	0	4.80	+546	0.61	.0576
Corn Meal	3	5.00	- 84	0.56	.0565
(Pot II)	4	4.70	+ 21	0.56	.0578
	9	4.70	-194	0.41	.0603
Soil	0	5.00	+496	0.70	.0516
(Pot III)	3	5.10	+236	0.47	.0551
	4	5.60	+ 96	0.50	.0513
	9	6.25	- 4	0.41	.0540
Soil	0	5.00	+546	0.50	.0456
(Pot IV)	3	4.98	+326	0.56	.0617
	4	5.90	+161	0.44	.0554
	9	6.31	+121	0.53	.0509

[1]Corn meal was added to the pots at the rate of 10 tons per acre.

Table 7. Effect of corn meal on fatty acid levels in Richard soil submerged under water in the greenhouse.

Treatment	Sampling (days after flooding	M.E. Fatty Acids[1]			
		Total	Acetic	Propionic	Butyric
Soil + Corn Meal[2] (Pot I)	0	.05366	.05042	.00224	.00090
	3	.13316	.08426	.00228	.04662
	4	.20716	.12582	.00350	.07784
	9	.06572	.05234	.00734	.07604
Soil + Corn Meal (Pot II)	0	.01518	.01424	.00094	.00000
	3	.13532	.08766	.00304	.04462
	4	.19060	.10426	.00354	.08280
	9	.20792	.11192	.01038	.08562
Soil (Pot III)	0	.00955	.00515	.00440	.00000
	3	.02496	.01782	.00134	.00610
	9	.07172	.05958	.00304	.00910
Soil (Pot IV)	0	.03446	.03288	.00158	.00000
	3	.04222	.03964	.00150	.00108

[1]Values are milliequivalents per 30 grams of water saturated soil.

[2]Corn meal was added to the pots at the rate of 10 tons per acre.

Table 8. Effect of corn meal on nematodes and bacteria in Richard soil submerged under water in the greenhouse.

Treatment	Sampling (days after flooding	Number of bacteria per gm of soil	Number of nemas per 1/7 pint of soil	Number of plant parasitic nemas per 1/7 pint of soil
Soil +	0	7.00×10^4	57.72	11.40
Corn Meal	3	7.72×10^5	82.00	7.50
(Pot I)	4	2.57×10^6	12.50	3.50
	9	2.57×10^6	2.00	0.00
Soil +	0	6.43×10^3	57.72	11.40
Corn Meal	3	7.72×10^5	29.50	4.50
(Pot II)	4	1.32×10^6	11.50	4.00
	9	2.57×10^7	1.50	0.00
Soil	0	4.00×10^5	57.72	11.40
(Pot III)	3	2.28×10^6	110.00	8.50
	4	2.28×10^7	106.00	12.00
	9	2.28×10^6	100.00	16.00
Soil	0	7.72×10^5	57.72	11.40
(Pot IV)	3	1.32×10^6	115.00	14.00
	4	4.00×10^6	90.00	7.50
	9	2.28×10^6	146.50	25.00

[1]Corn meal was added to the pots at the rate of 10 tons per acre.

Table 9. Fatty acids in the flood water in rice fields in 1963.

Sampling Site	Sampling (days after flooding)	M.E. Fatty Acids [1]				pH
		Total	Acetic	Propionic	Butyric	
Compton	71	.02854	.02600	.00147	.00107	6.50
	78	.03022	.02800	.00104	.00118	--
	85	.02208	.02111	.00097	.00000	--
Davis	52	.03659	.03509	.00150	.00000	6.85
	59	.03528	.03055	.00150	.00323	6.50
	66	.03854	.03854	.00000	.00000	--
	73	.02998	.02764	.00150	.00084	--
Richard	89	.03210	.03074	.00136	.00000	6.72
Petitjean	20	.04038	.03656	.00278	.00104	7.40
	34	.02052	.01860	.00192	.00000	7.20
	41	.03708	.03516	.00192	.00000	--
	48	.02008	.01878	.00130	.00000	--
Wild	15	.03206	.03088	.00118	.00000	6.90
	22	.02518	.02368	.00118	.00032	6.75
	43	.03221	.03104	.00117	.00000	6.70
	50	.01153	.01053	.00100	.00000	--
Fogelman	16	.03635	.03458	.00177	.00000	6.80
	23	.02421	.02288	.00133	.00000	6.50
	30	.02897	.02729	.00168	.00000	--
	37	.02702	.02525	.00177	.00000	--
Byler	72	.03108	.02949	.00133	.00026	6.40

[1]Values in this table are averages of two samples each and are expressed in milliequivalents per pint of water (473.2 ml).

Table 10. Effects of sampling depth on physical variables in flooded rice fields in 1963.

Sampling Site	Sampling (days after flooding)	Soil depth (inches)	pH	Eh (millivolts)
Petitjean	48	0.0-0.5	7.15	- 47
	48	0.0-0.5	6.70	-114
	48	0.5-4.3	7.10	+ 51
	48	0.5-4.3	6.80	- 36
Wild	50	0.0-0.5	6.20	- 65
	50	0.0-0.5	6.50	- 54
	50	0.5-4.3	6.60	- 83
	50	0.5-4.3	6.60	- 74
Fogelman	37	0.0-0.5	6.40	-155
	37	0.0-0.5	6.15	- 94
	37	0.5-4.3	6.70	- 24
	37	0.5-4.3	6.80	- 20
Compton	85	0.0-0.5	6.60	- 74
	85	0.0-0.5	6.50	- 54
	85	0.5-4.3	6.70	- 77
	85	0.5-4.3	6.50	- 44
Davis	73	0.0-0.5	6.55	+ 31
	73	0.0-0.5	6.90	- 59
	73	0.5-4.3	6.40	- 76
	73	0.5-4.3	6.30	- 29

Table 11. Effect of sampling depth on fatty acids in flooded rice fields in 1963.

Sampling Site	Sampling (days after flooding)	Soil depth (inches)	M.E. Fatty Acids[1] Total	Acetic	Propionic	Butyric
Petitjean	48	0.0-0.5	.03694	.03310	.00292	.00092
	48	0.0-0.5	.01193	.00455	.00660	.00078
	48	0.5-4.3	.04316	.04068	.00156	.00092
	48	0.5-4.3	.03676	.03380	.00192	.00104
Wild	50	0.0-0.5	.00212	.00202	.00008	.00002
	50	0.0-0.5	.00073	.00070	.00003	.00000
	50	0.5-4.3	.00082	.00075	.00005	.00002
	50	0.5-4.3	.00255	.00214	.00041	.00000
Fogelman	37	0.0-0.5	.04380	.04034	.00230	.00116
	37	0.0-0.5	.03624	.03254	.00354	.00116
	37	0.5-4.3	.02532	.02338	.00194	.00000
	37	0.5-4.3	.00856	.00712	.00106	.00038
Compton	85	0.0-0.5	.02100	.02100	.00000	.00000
	85	0.0-0.5	.03866	.03866	.00000	.00000
	85	0.5-4.3	.02406	.02234	.00172	.00000
	85	0.5-4.3	.00966	.00966	.00000	.00000
Davis	73	0.0-0.5	.03602	.03236	.00366	.00000
	73	0.0-0.5	.05280	.04836	.00216	.00228
	73	0.5-4.3	0.3104	.02800	.00184	.00120
	73	0.5-4.3	.02500	.02182	.00150	.00168

[1]Values in this table are expressed in milliequivalents per 30 grams of water saturated soil.

Table 12. Effect of temperature on concentrations of fatty acids in saturated Wild soil. [1]

Treatment	Replicate	Temperature (°C)	M.E. Fatty Acids[2] Total	Acetic	Propionic	Butyric
Soil	1	23	.02592	.02500	.00092	.00000
Soil	1	30	.02076	.01914	.00102	.00060
Soil + acids	1	30	.08212	.03276	.04936	.00000
Soil	2	23	.03229	.02942	.00156	.00131
Soil + acids	2	23	.01449	.06340	.05140	.02810
Soil	2	30	.02232	.02144	.00088	.00000
Soil + acids	2	30	.06700	.03362	.03338	.00000

[1] Soil was taken from the field 15 days after flooding and 4 m.e. each of formic, acetic, propionic, and butyric acids were added per pint of saturated soil and incubated at indicated temperatures for 9 days.

[2] Values are milliequivalents per 30 grams of water waturated soil.

Table 13. Effect of temperature on concentrations of fatty acids in saturated Richard soil.[1]

Treatment	Replicate	Temperature (°C)	M.E. Fatty Acids[2]			
			Total	Acetic	Propionic	Butyric
Soil	1	23	.04538	.04156	.00292	.00090
Soil + acids	1	23	.17218	.05600	.05630	.05988
Soil	1	30	.03342	.02840	.00264	.00238
Soil + acids	1	30	--	--	--	--
Soil	2	23	.04022	.03800	.00222	.00000
Soil + acids	2	23	--	--	--	--
Soil	2	30	.03498	.03130	.00308	.00060
Soil + acids	2	30	.13926	.05498	.06162	.00286

[1] Soil was taken from the field 89 days after flooding and 4 m.e. each of formic, acetic, propionic, and butyric acids were added per pint of saturated soil and incubated at indicated temperatures for 8 days.

[2] Values are milliequivalents per 30 grams of water saturated soil.

Table 14. Effect of temperature and associated factors on meatode populations in saturated Wild soil.[1]

Treatment	Replicate	Temperature (°C)	Number of total nemas per 1/7 pint of soil	Number of plant parasitic nemas per 1/7 pint of soil
Soil	1	23	129	18
Soil + acids	1	23	111	5
Soil	1	30	45	10
Soil + acids	1	30	91	4
Soil	2	23	174	3
Soil + acids	2	23	183	4
Soil	2	30	127	4
Soil + acids	2	30	155	6

[1] Soil was taken from the field 15 days after flooding and 4 m.e. each of formic, acetic, propionic, and butyric acids were added per pint of saturated soil and incubated at indicated temperatures for 9 days.

Table 15. Effect of temperature and associated factors on nematode populations in saturated Richard[1] soil.

Treatment	Replicate	Temperature (°C)	Number of total nemas per 1/7 pint of soil	Number of plant parasitic nemas per 1/7 pint of soil
Soil -	1	23	8	0
Soil + acids	1	23	2	0
Soil	1	30	26	16
Soil + acids	1	30	28	10
Soil	2	23	48	0
Soil + acids	2	23	20	8
Soil	2	30	18	2
Soil + acids	2	30	10	0

[1]Soil was taken from the field 89 days after flooding and 4 m.e. each of formic, acetic, propionic and butyric acids were added per pint of saturated soil and incubated at indicated temperatures for 8 days.

Table 16. Total nematodes per 1/7 sample aliquot (approx. 30 ml) from single 10 x 10 feet plot in Richard rice field (6 replicates of 200 ml soil samples taken 0-4.3 in. depth at intervals during 1964).

Sampling Date	Replicate 1	2	3	4	5	6	Mean
25 Mar.	260	180	220	132	200	157	192
2 Apr.	132	230	287	140	320	212	220
13 May	128	132	127	120	125	128	127
20 May	280	322	208	260	520	340	322[1]
1 June	160	250	175	160	105	55	151
12 June	116	120	160	124	140	84	124
23 June	84	55	30	120	70	65	71
29 June	56	40	38	60	42	40	46
13 July	13	26	40	15	31	18	24
24 July	38	30	46	40	26	10	32
7 Aug.	6	5	6	13	12	10	9

[1] Difference between mean of fourth sampling (20 May) and each of the other means exceeded the "shortest significant range" (Rp) for each value of p at the 1 per cent level in Duncan Range Test (32).

Table 17. The oxygen level[1] in terms of oscilloscope value in mm of flooded Wild soil planted to rice and incubated in the greenhouse. The effect of depth and time of submergence.

Days after flooding	Soil depth (cms)									
	0		3		5		9.5		15	
	Oscilloscope readings in mm of duplicate pairs of electrodes									
3	28	32	30	31	32	33	36	38	39	40
4	43	41	-	46	42	46	41	49	55	55
11	30	37	-	49	57	47	41	46	42	48
13	29	28	-	35	51	42	45	37	37	-
14	29	28	-	48	46	43	42	44	39	70
15	29	31	-	47	46	44	42	44	37	66
25	29	31	-	48	50	49	45	46	44	58
26	38	38	-	47	52	39	40	42	37	56
27	44	45	-	55	52	41	45	50	46	61
28	40	33	-	53	57	38	49	47	45	74
49	37	43	-	51	50	44	43	-	53	-
50	35	41	-	51	50	44	43	-	53	-

[1]Oxygen concentration is inversely proportional to oscilloscope value. Oxygen is oxygen-saturated water (8 ppm) equals 43 mm oscilloscope reading; 0 ppm oxygen equals 76 mm in nitrogen-saturated water.

[2]Readings represent diffusion current (i) at the oxygen maxima. This is expressed as deflection (in mm) on the oscilloscope screen caused by the voltage drop at 27°C. i is proportional to the oxygen concentration in the soil, in the immediate vicinity of the electrodes.

[3]Values 58 to 74 reflect very low oxygen due to the absence of roots.

DISCUSSION

Anaerobic conditions established by the flooding of rice soils favor the development of anaerobic bacteria that produce lower organic acids as end products. Amounts of these acids in a soil will be dependent not only on the organic matter content but also on the kind of organic materials. Sugars and starches will be more readily decomposed by these bacteria than cellulose or lignin (77). It is only in certain organic soils that considerable amounts of easily oxidizable organic materials are present and it is in these soils that the production of lower organic acids results in deleterious effects on rice plants. Of the organic acids produced during the anaerobic decomposition of fermentable organic matter, butyric acid is perhaps the most important. It is present in appreciable amounts in the early stages of decomposition, may persist in acid soils, and is toxic to plants (39, 40). Mitsui and co-workers (39) have shown that butyric acid at less than 0.1 mol per liter can cause a marked decrease in nutrient uptake. Failure of rice seedlings on newly manured fields could be related to toxic effects of butyric acid and carbon dioxide (48).

Rice soils in Louisiana are not rich in fermentable materials because of their rapid decomposition under well aerated conditions between crops (2). These soils consequently are not capable of sustaining large populations of

clostridial bacteria producing butyric and other lower fatty acids. A short period of activity by these bacteria immediately after flooding results in an increase in the lower fatty acids, which is soon arrested when the supply of fermentable organic matter is exhausted in soil. This explains the rapid increases in hydrion concentration, the decrease in oxidation-reduction potentials, as well as the initial peak values of fatty acids obtained in the first 15-20 days after submergence. The limited supply of easily oxidizable organic matter in these soils is also reflected in the relatively constant levels of organic carbon throughout the rice season. A soil rich in such organic materials should show detectable decreases in organic carbon provided available nitrogen is sufficient for maintenance of favorable carbon-nitrogen ratios.

Decline in acid concentrations in the soil after the "early season" peak values could be related to:

a. A depression in the numbers of fatty acid producing bacteria when the supply of fermentable organic matter is exhausted. This is suggested by the decline in numbers of anaerobic spore forming bacteria observed in all soils after 15 days of submergence.

b. A gradual seepage of organic acids into the flood water of rice fields. This could explain the presence of butyric, propionic and acetic acids in these waters.

c. The development of rice roots in the soil. Oxygen around the roots could depress populations of strict anaerobes (59, 76). However, root systems of rice plants are not well developed at the early stage of growth.

Eh values taken early in the season indicate reduced conditions, which would only rise later in the season with the release of increased amounts of oxygen from extensive root systems.

d. Utilization of the acids by other microorganisms (63, 64). This would be particularly true late in the season when hydrion concentrations are favorable for the development of Desulfovibrio sp. (64). Takai (64) showed an inverse relationship between numbers of bacteria in deep tube culture and numbers of sulfate reducing bacteria (Desulfovibrio sp.) which appeared in large numbers late in the season in Japanese soils.

A combination of (a) and (b) could account for the initial decline in acid values, early in the season, and (c) and (d) might play an important role in the decline of acid concentrations after the late season peak.

The presence of fatty acids throughout the season contrasts directly with the results obtained by Takai (66, 67), who found that these acids disappeared from Japanese rice soils after an initial rise in their concentration immediately after submergence. This apparent discrepancy may be explained by the fact that organic matter decomposition occurs at a faster rate under Louisiana conditions than in the more temperate climate of Japan. This would afford a continuous production of acids from cellulose and similar materials by cellulose-decomposing clostridia. The amounts of acids produced by these bacteria is considerably less than those produced by the butyric group of clostridia (5). Since studies of cellulose-decomposing bacteria were not made in this work, this explanation is tentative.

The late season peak in acid values observed in most soils suggests a change in the soil microflora. A group of microorganisms capable of utilizing more resistant forms of organic matter could bring about an increase in acids. Organic debris from the rice plants might favor increased activity of anaerobic bacteria later in the growing season. The final increase and decline of acids during the rice growing season is typical of Louisiana rice soils; moreover, it is probably typical of subtropical soils around the world which are commonly low in total nitrogen and organic carbon.

The predominance of acetic acid over all other acids in the 1963 plots was expected, since this acid is produced in greater quantitites by anaerobic bacteria. Some anaerobic bacteria are incapable of producing butyric acid and acetic acid is the only fatty acid end-product of their metabolism.

The greater decrease in concentrations of butyric and propionic acids over those of acetic acid, when these acids were added to the soil, indicates a possible conversion of the heavier acids into acetic acid. The close statistical correlations obtained when acetic acid was compared with propionic and butyric acids, and the presence of butyric acid, in the 1963 samples only when acetic acid was relatively high in concentration, is of interest in this connection. The interrelations among these acids and their biological sources furnish a problem amenable to labeling of compounds with radioactive carbon. The metabolism of butyric and propionic acids by soil microorganisms is of importance relative to nematodes because these two acids are nematicidal at

relatively low concentrations (28, 29). Acetic acid kills nematodes only at high concentrations.

The occurrence of low concentrations of fatty acids composed primarily of acetic acid in rice fields results when only limited amounts of readily fermentable organic matter are present. Results of the 1963 greenhouse experiment on the effect of corn meal indicate another important and totally different situation. The pronounced increase in concentrations of propionic and butyric acids in this latter case contrasts with the low levels of these acids in rice fields. This is connected with a different type of fermentation in which butyric acid bacteria predominate when corn meal is present.

Nematicidal effects of the lower organic acids afforded an initial explanation for the decline of nematodes in rice fields (28). However, a material which is effective in the laboratory may not be effective in the soil because: a. it may be inactivated by other soil microorganisms, b. colloidal particles may absorb it, c. inactivation by soil chemicals may occur, d. an energy source may be lacking, and e. the toxic material may not be produced in large enough quantities (77). These factors obviously were absent in this work because the phenomenon investigated actually occurred in rice fields. It is apparent that the effect of flooding on nematodes must be considered under two categories: In soils high in readily decomposable organic matter the rapid decline of nematode populations will be due in part to butyric and propionic acids in nematicidal concentrations. In soils poor in readily fermentable organic matter the slow decline of nematode populations is linked to the production of hydrogen

sulfide. In soils higher in fermentable organic matter the decline of nematode populations is correlated with the presence of high concentrations of fatty acids and sulfides.

The presence of lower fatty acids in rice flood waters has not been previously reported. The results obtained by sampling at different depths in rice soils indicate that acetic, propionic and butyric acids move upward through the soil into the flood waters above. Concomitant fluctuations in acid concentrations in flood waters and underlying soils serve as additional evidence for their origin. The presence of these acids in flood waters may be of importance as an ecological factor to wild life since these waters are discharged into canals and rivers.

Sulfides are invariably present in soils which have been under water for an appreciable length of time. They are the result of a small group of bacteria using sulfate as their source of energy. The presence of sulfides in rice fields has been repeatedly demonstrated (17, 35, 39, 40, 42, 43, 44, 60, 63, 64, 65, 68, 69). Hydrogen sulfide in rice soils raises the problem of its toxicity. This gas is an inhibitor of the iron-containing enzymes, cytochrome oxidase, catalase, and peroxidase, and is consequently toxic to plants. The formation of insoluble sulfides with heavy metals inhibits metabolic reactions in which these metals act as co-factors to the enzymes involved. Hydrogen sulfide is a strong toxicant to higher animals since it combines with methemoglobin which causes asphyxia and sudden death if present in sufficient amounts (11).

The deleterious effects of hydrogen sulfide on rice plants are counteracted in rice fields by the presence of ferrous iron (35) and subsequent formation of a black layer of ferrous sulfide near the soil surface (4). The action of hydrogen sulfide on rice plants has been studied extensively in Japan by Mitsui and coworkers (38, 39). in connection with the occurrence of the Akiochi disease of rice in some degraded soils. Rice plants subject to this disease show a vigorous growth in the early stages, but the plants are very often severely attacked by the rice blast fungus Piricularia oryzae Briosi and Cavara). As a result, leaves die one by one, beginning with the lower ones. Plants exhibit a miserable appearance at harvest time and the yields are low (10). The roots of diseased plants are white in contrast to the reddish brown color of healthy roots (39). This disease occurs only in soils depleted of iron, such as in certain sandy soils of Japan. The application of materials containing iron and the elimination of ammonium sulfate as a fertilizer have resulted in the control of this disease (48).

The effect of hydrogen sulfide on rice plants was studied by Mitsui under laboratory conditions (38). This worker found that exposure of rice roots to a solution containing .07 ppm of hydrogen sulfide for 9 days was sufficient to cause wilting. He found also that hydrogen sulfide injury was a function of its concentration in the solution around the roots and the time of exposure of the plants to it. Hydrogen sulfide also inhibited the accumulation of phosphates, potassium and silica.

The occurrence of the Mentek disease of rice in Java (73) has been

related to the presence of reduced products in rice soils (48, 60). It is possible that hydrogen sulfide in rice fields is connected with this disease, as well as to other physiological disorders of rice throughout the world.

The action of hydrogen sulfide on nematodes can be considered as an extension of its action against other organisms. The blackening of the nematode body, upon exposure to this compound, suggests the formation of metal sulfides which precipitate and interfere with the respiratory functions of these animals. The continuous increase in levels of hydrogen sulfide in Louisiana rice fields during the rice season is linked to the slow decline of nematode populations in these fields. A similar compound HCN, produced by an unidentified low-temperature basidiomycete has been implicated as an etiological agent in winter crown rot of alfalfa (30); there is also some evidence that the lesion nematode P. penetrans (Cobb, 1917) Filip. and Stek 1941, induces the formation of HCN in peach roots thus causing the formation of lesions (41). However, H_2S is the first case reported of a compound antibiotic to plant parasites under laboratory conditions which has also been found antibiotic in soil under actual field conditions. The importance of this discovery prompts questions regarding field conditions and practices that would enhance the development of sulfides, i.e., what practices will favor the development of sulfate reducing bacteria in rice fields?, what levels of hydrogen sulfide can rice plants withstand under actual field conditions without visible damage?, how can nematicidal concentrations of hydrogen sulfide be maintained throughout the season without injury to rice plants?, what are the deleterious effects caused

by sulfides and hydrogen sulfide on the nutrition of the rice plant?, what are the effects of the electrical fields created by the sulfate reducing bacteria in the movement of nutrients to rice roots (47)? The availability of soil nutrients in connection with development of large populations of Desulfovibrio sp. in rice soils present many interesting questions regarding their distribution in rice fields and their ability to withstand relatively high concentrations of oxygen (47, 57).

Oxygen levels in rice soil suggest this gas could exert a depressing effect on soil anaerobiosis. The environment around rice roots is aerobic and therefore may be relatively free of anaerobic microorganisms; this could also account for the survival of small populations of nematodes under rice field conditions. Such activity of the rice roots would be more pronounced on anaerobic bacteria, other than Desulfovibrio sp., due to the ability of this bacterium to "synthesize" an anerobic environment around its colonies (47, 57). T. martini and possibly other nematodes are capable of withstanding zero oxygen tensions for periods of at least 2 weeks, as determined by in vitro tests in the course of this research. The combined evidence indicates that carbon dioxide, hydrogen and methane are without direct effects on nematode populations in submerged soils, although carbon dioxide might render nematodes more sensitive to hydrogen sulfide.

SUMMARY

Effects of microbiological and chemical variables on soil nematode populations in flooded rice fields were studied in 13 field sites in Louisiana in 1963 and 1964 and in several laboratory experiments. Percentage organic carbon (0.9-1.3) and total nitrogen (0.09-0.16) reflect typical levels for rice soils in subtropical regions of the world.

1. Laboratory experiments showed that hydrogen sulfide was antibiotic to nematodes at concentrations as low as 10 ppm. The action of hydrogen sulfide against nematodes was shown to be dependent on time of exposure and concentration.

2. Concentrations of hydrogen sulfide in rice fields increased from 0.1 ppm five to seven days after flooding to levels varying from 10 to 45 ppm at the end of the season.

3. Decline in nematode numbers was related inversely to the rise of hydrogen sulfide concentrations throughout the season.

4. Plant parasitic nematodes were more susceptible to the action of hydrogen sulfide than all types of nematodes combined.

5. Colonies of sulfate reducing bacteria (Desulfovibrio sp.) were isolated from rice fields.

6. The presence of hydrogen sulfide in rice fields is connected with the activities of sulfate reducing bacteria, the action of organic acids on sulfides and the anaerobic decomposition of proteins.

7. The action of weak organic acids on insoluble sulfides was demonstrated in the laboratory to provide a mechanism for the continuous production of hydrogen sulfide; this was postulated to occur in rice fields.

8. These results afford the first example of a broad-scale field control of nematodes by antibiosis.

9. Nematodes were not affected by the absence of oxygen when exposed to atmospheres of hydrogen, nitrogen and carbon dioxide.

10. Oxygen supplied to the soil by ricc roots creates aerobic conditions in the anaerobic environment that may serve to maintain nematode populations, depress the activity of anaerobic bacteria other than Desulfovibrio sp., and interfere with the biological effects of hydrogen sulfide on nematodes and other organisms.

11. Acetic, propionic and butyric acids were present throughout the season in rice fields. Their concentrations averaged in m.e. per 30 gms of water saturated soil: acetic 0.03, propionic 0.002, and butyric 0.0009. Formic acid was absent in rice fields.

12. Concentrations of acids were highest 15 to 20 days after submergence. These levels declined progressively to increase again late in the season and finally show a decline before harvest.

13. Lower fatty acids were not effective against nematodes at the levels and hydrion concentrations at which they occurred in rice fields.

14. Soils amended with easily decomposable organic matter (corn meal) in the laboratory, produced butyric and propionic acids at concentrations which

rapidly killed nematodes after 9 days of submergence. This is postulated to occur in soils rich in fermentable organic matter. These soils are not found in the Louisiana rice area.

15. Correlation coefficients between concentrations of acetic and propionic, acetic and butyric acids in rice fields were highly significant at the 1 per cent level of probability, suggesting possible interconversions of the acids.

16. The decline in concentrations of acetic, propionic and butyric acids, when added to saturated rice soils, was directly related to their molecular weights.

17. Organic acids were in higher concentrations at 0 to 1/2 inch than at 1/2 to 4.3 inches soil depth in rice fields.

18. Organic acids in the flood waters of rice fields were those present in the soil, at approximately 1/15 of the soil concentrations, and the evidence suggests that they originated from the soil by diffusion into the water.

18. Five species of clostridia were isolated from rice soils consistently throughout the season:

Cl. acetobutylicum.

Cl. amylosaccharobutylpropylicum.

Cl. butylicum.

Cl. butyricum.

Cl. pasteurianum.

Clostridium roseum was isolated only once from one field.

LITERATURE CITED

1. Acharya, C. N. 1935. Studies on the anaerobic decomposition of plant materials I. Anaerobic decomposition of rice straw. Biochem. J. 29:528-541.

2. Alexander, M. 1961. Introduction to soil microbiology. John Wiley and Sons, Inc. New York.

3. Allen, O. N. 1953. Experiments in soil bacteriology. Burgess Publ. Co., Minneapolis. 127 p.

4. Aomine, S. 1962. A review of research on redox potentials of paddy soils in Japan. Soil Science 94: 6-13.

5. Barker, H. A. 1956. Bacterial fermentations. CIBA. Lectures in microbial biochemistry. John Wiley and Sons, Inc. New York, 95 pp.

6. Bessey, E. A. 1911. Root-knot and its control. U. S. Dept. Agri., Bureau Plant Industry. Bull. 217. 89 pp.

7. Breed, R. S., E. D. G. Murray and A. P. Hitchens. 1957. Bergey's manual of determinative bacteriology. Williams and Williams Co., Baltimore. 1094 pp.

8. Brown, L. N. 1933. Flooding to control root-knot nematodes. J. Agr. Res. 47: 883-888.

9. Burstone, M. S. 1962. Enzyme histochemistry. Academic Press. New York.

10. Dion, H. G. 1953. F. A. O. development paper No. 39.

11. Fairhall, L. T. 1957. Industrial toxicology. The Williams and Wilkins Co., Baltimore. 375 pp.

12. Feldmesser, J. 1954. Some effects of aerated oxygen tensions on certain plant-parasitic and soil-inhabiting nematodes in vitro. J. Parasitology (Abs.). 40: 18.

13. Frandsen, P. 1916. Eelworm parasites of plants. Monthly Bull. Calif. State Commission of Hort. 5: 60-63.

14. Gehrke, C. N. and W. M. Lamkin. 1961. Quantitative determination of steam-volatile fatty acids by gas-liquid chromatography. Agr. and Food Chem. 9: 85-88.

15. Halvorson, H. O. and N. R. Ziegler. 1933. Application of statistics to problems in bacteriology. I. A means of determining bacterial populations by the dilution methods. J. Bacteriol. 25: 101-121.

16. Halvorson, H. O. and N. R. Ziegler. 1938. Quantitative bacteriology. Burgess Publ. Co., Minneapolis. 64 p.

17. Harrison, W. H. and S. Aiyer. 1913. The gases of swamp rice soils: Their composition and relation to the crop. India Dept. Agr., Mem. Chem. Ser. 3: 65-106.

18. Hamala, M., J. Marek and Z. Valcikova. 1955. Pouziti Polarografickych metod pro rozbor vod naftovych lozisck. Stanoveni nekterych aniontu (jodidu, bromidu a sioniku). Prace Ustavu P ro Naftovy Vyzkum. Publicace C. 4-8. Praha, 111-123 p.

19. Hollis, J. P. and T. Johnston. 1957. Microbiological reduction of nematode populations in water-saturated soils (Abs.) Phytopathology 47: 16.

20. Hollis, J. P., and M. J. Fielding. 1958. Population behavior of plant parasitic nematodes in soil fumigation experiments. La. Agr. Exp. Sta. Bul. 515. 30 p.

21. Hollis, J. P. 1958. Induced swarming of a nematode as a means of isolation. Nature 182: 956-957.

22. Hollis, J. P. 1960. Mechanism of swarming in Tylenchorhynchus species (Nematoda, Tylenchida). Phytopathology 50: 639.

23. Hollis, J. P. 1960. Reactions of soil nematodes to coal-tar dyes. Nature 188: 1128-1129.

24. Imamura, S. 1931. Nematodes in the paddy field, with notes on their populations before and after irrigation. J. Coll. Agric. Tokyo 11: 198-240.

25. Jackson, M. L. 1958. Soil Chemical Analysis. Prentice Hall, Inc., New Jersey, 498 pp.

26. Johnston, T. 1957. Further studies on microbiological reduction of nematode population in water-saturated soils (Abs.). Phytopathology 47: 525.

27. Johnston, T. 1958. The effect of soil moisture on Tylenchorhynchus martini and other nematodes. Proc. La. Acad. Sci. 20: 52-55.

28. Johnston, T. 1958. Antibiosis of Clostridium butyricum Prazmowski on Tylenchorhynchus martini Fielding 1956, (Nematoda, Phasmidia) in submerged rice soil. A thesis. La. State Univ. 62 pp.

29. Johnston, T. 1959. Effect of fatty acid mixtures on the rice stylet nematode (Tylenchorhynchus martini Fielding, 1956). Nature 183: 1392.

30. Lebeau, J. B. and J. G. Dickson. 1955. Physiology and nature of disease development in winter crown rot of alfalfa. Phytopathology 45: 667-673.

31. Lebert, F., and P. Tardieux. 1952. Technique d'isolement et de determination des bacteries anaerobies. Pacomhy, S.A. R.L. 2nd. ed., Paris. 55 pp.

32. Leclerg, E. C., W. H. Leonard and A. G. Clark. 1964. Field plot technique. Burgess Publ. Co., Minneapolis. 144 pp.

33. Lemon, E. R. and A. E. Erickson. 1955. Principle of the platinum microelectrode as a method of characterizing soil aeration. Soil Sci. 79: 383-392.

34. Lingane, J. J. 1961. Chronopotentiometric study of oxygen reduction at a platinum wire cathode. J. Electroanal. Chem. 2: 316-319.

35. Mandal, L. N. 1961. Transformation of iron and manganese in water-logged rice soils. Soil Sci. 91: 121-126.

36. Millar, C. E. 1955. Soil Fertility. John Wiley and Sons, Inc., New York, 436 pp.

37. Mitsui, S., et al. 1949. Nature of akiochi (autumn-decline of rice plants) and its amelioration. Agriculture and Horticulture 24: 173-176.

38. Mitsui, S., S. Aso and K. Kumazawa. 1951. Dynamic studies on nutrient uptake by crop plants. Part I. The nutrient uptake of rice root as influenced by H_2S. J. Sci. Soil Man. 22: 46-52.

39. Mitsui, S., K. Kumazawa and T. Ishiwara. 1953. Dynamic studies on the nutrient uptake by crop plants I. Effect of butyric acid and respiration inhibitors such as H_2S, NaCn and NaN_3 on the nutrient uptake by rice soil. J. Sci. Soil and Man. 24: 45-50.

40. Mitsui, S., K. Kumazawa and N. Mukai. 1959. Dynamic studies on the nutrient uptake by crop p[illegible]. XXII. The growth of rice plant on poorly drained soil as affected by accumulation of volatile organic acids. I. J. Sci. of Soil and Manure, Japan 30: No. 7: 329-373.

41. Mountain, W. B. and Z. A. Patrick. 1959. The peach replant problem in Ontario VII. The pathogenicity of Pratylenchus penetrans (Cobb, 1917) Filip. & Stek. 1941. Can. J. Botany 37: 459-470.

42. Osugi, S. 1934. On the peculiar acid soils. J. Sci. Soil Mature, Japan 8 (supplement): 75-76.

43. Osugi, S., Aoki, M., and S. Morita. 1934. On the peculiar acid soils I. J. Agr. Chem. Soc., Japan 10: 443-450.

44. Osugi, S., Aoki, M., and K. Kawaguchi. 1938. On the cause of physiological disease with applied ammonium sulfate. J. Sci. Soil Manure, Japan 13: 1-10.

45. Parker, C. A. 1955. Anaerobiosis with iron wool. Aust. J. Exptl. Biol. Med. Sci. 33: 33-37.

46. Piper, C. S. 1950. Soil and plant analysis. Interscience Publishers, Inc., 368 pp.

47. Pochon, J., and H. de Barjac. 1958. Traite de microbiologie des sols. Dunod. Paris.

48. Ponnamperuma, F. N. 1955. The chemistry of submerged soils in relation to the growth and yield of rice. Thesis, Cornell Univ. 208 p.

49. Prevot, A. R. 1948. Manuel de classification et de determination des bacteries anaerobies. Masson et Cie., 2nd ed., Paris.

50. Raalte, M. H. Van. 1940. On the oxygen supply of rice roots. Ann. Jard. Bot. Buitenzorg 50: 99.

51. Rodriguez-Kabana, R., and J. W. Jordan. 1964. A mechanism for continuous production of hydrogen sulfide in soils submerged in water. Phytopathology 54: 887.

52. Rodriguez-Kabana, R., J. W. Jordan, and J. P. Hollis. 1964. Biological control of nematodes in rice fields: the role of hydrogen sulfide. Science, in Press.

53. Russel, E. J. 1961. Soil conditions and plant growth. 9th. ed. Longmans. London.

54. Schwartz, S. M , J. E Varner and W. P. Martin. 1954. Separation of organic acids from several dormant and incubated Ohio soils. Soil Sci. Soc. Proceedings 18: 174-177.

55. Seinhorst, J. W. Personal communication.

56. Society of American Bacteriologists. 1957. Manual of Microbiological Methods. McGraw-Hill Book Co., Inc., 315 pp.

57. Starkey, R. L. 1958. The general physiology of the sulfate reducing bacteria in relation to corrosion. Producers Monthly, 22(9): 12-36.

58. Stolzy, L. H., S. D. Van Gundy, C. K. Labanauskas, and T. E. Szwozkiewicz. 1953. Response of Tylenchulus semipenetrans infected citrus seedlings to soil aeration and temperature. Soil Science 96: 292-298.

59. Stover, R. H. 1962. Fusarial wilt (Panama disease) of bananas and other Musa species. The Commonwealth Mycological Institute, Kew, Surrey.

60. Sturgis, M. B. 1936. Changes in the oxidation-reduction equilibrium in soils as related to the physical properties of the soil and the growth of rice. La. State Univ. Ag. Expt. Sta. Bull. 271.

61. Subrahmanyan, V. 1927. Biochemistry of waterlogged soils. I. The effect of waterlogging on different forms of nitrogen, on the reaction, on gaseous relationships and on bacterial flora. J. Agr. Sci. 17: 429-448.

62. Subrahmanyan, V. 1929. Biochemistry of waterlogged soils: III. J. Agr. Sci. 19: 627-648.

63. Takai, T., T. Koyama and T. Kamura. 1955. Microbial metabolism of paddy soils. J. Agr. Chem. Soc., Japan 29: 967-972.

64. Takai, T., T. Koyama and T. Kamura. 1956. Microbial metabolism in reduction processes of paddy soils: I. Soil and Plant Food 2: 63-66.

65. Takai, T., T. Koyama and T. Kamura. 1957. Microbial metabolism of paddy soils. J. Agr. Chem. Soc., Japan 31: 211-220.

66. Takai, T. 1958. On the quantitative analysis of organic acids in paddy soils. I. Quantitative analysis of organic acids by silica gel column chromatography, and separation of organic acids in soil by ether extraction. J. Sci. Soil Manure, Japan 28(No. 11): 7-10.

67. Takai, T. 1958. On the quantitative analysis of organic acids in paddy soils. II. Separation of soil organic acids by the use of ion exchangers. J. Sci. Soil Manure, Japan (No. 2): 47-86.

68. Takai, T. 1961. The reduction of paddy soil and microbial metabolism. Technics for Agriculture (Nogyu-Gijutsu) 16: 213-216.

69. Takai, T., and T. Asami. 1962. Formation of methyl mercaptan in paddy soils. I. Soil Sci. and Plant Nutrition 8 (No. 3): 40-44.

70. Tarjan, A. C., and P. C. Cheo. 1956. Nematocidal value of some fatty acids. Univ. Rhode Island Exp. Sta. Bull. 332. 41 pp.

71. Tarjan, A. C., and P. C. Cheo. 1956. The nematocide screening program of the University of Rhode Island. Univ. Rhode Island Mis. Pub. 47. 27 pp.

72. Treadwell, F. P., and W. T. Hall. 1937. Analytical Chemistry. Vol. I. John Wiley and Sons, Inc., 630 pp.

73. Van der Vecht, J. 1953. The problem of the mentek disease of rice in Java. Pemb. Balai Besar Penj. Pert. No. 137: 1-88.

74. Van Gundy, S. D., and L. H. Stolzy. 1961. Influence of soil oxygen concentrations on the development of Meloidogyne javanica. Science 134: 665-666.

75. Van Gundy, S. D., L. H. Stolzy, T. E. Szusykiewicz, and R. L. Rackham. 1962. Influence of oxygen supply on survival of plant parasitic nematodes in soil. Phytopathology 52: 628-632.

76. Vlamis, J., and A. R. Davis. 1944. Effects of oxygen tension on certain physiological responses of rice, barley, and tomato. Plant Physiol. 19: 33.

77. Waksman, S. A. 1952. Principles of soil microbiology. John Wiley and Sons, Inc. New York. 356 pp.

78. Wallace, H. R. 1964. The biology of plant parasitic nematodes. St. Martin's Press, Inc. New York. 280 pp.

79. Watson, J. R. 1921. Control of root-knot II. Fla. Agr. Exp. Sta. Bull. 159, pp. 31-44.

80. Willey, C. R., and C. B. Tanner. 1963. Membrane-covered electrode for measurement of oxygen concentration in soil. Soil Sci. Soc. Am. Proc. 27: 511-515.

81. Winogradsky, S. 1949. Microbiologie du Sol. Masson et Cie. Paris. 861 pp.

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Title of Thesis: Chemical Antibiosis to Nematodes in Rice Fields

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